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Emerging Foodborne Pathogens: *Escherichia coli* O157:H7 as a Model of Entry of a New Pathogen into the Food Supply of the Developed World

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INTRODUCTION

History

In 1982, an investigation by the Centers for Disease Control and Prevention (CDC) of two outbreaks of severe bloody diarrhea, associated with the same fast-food restaurant chain, led to the identification of a strain of *Escherichia coli*, one that expressed O-antigen 157 and H-antigen 7, that had not previously been recognized as a pathogen (1, 2). Subsequently, this strain was shown to belong to a category of *E. coli* that produce toxins which are similar to Shiga toxin of *Shigella dysenteriae* and distinct from previously described *E. coli* heat-stable and heat-labile toxins. As data were accumulating on the role of *E. coli* O157:H7 as a pathogen, parallel work in Canada was uncovering high rates of infection with this and other Shiga toxin-producing *E. coli* in patients with the hemolytic uremic syndrome (3, 4). Subsequent research has indicated that *E. coli* O157:H7 is the cause of 85–95 percent of cases of hemolytic uremic syndrome in North America, and that non-O157 Shiga toxin-producing *E. coli* are responsible for another 5–15 percent (5).

In the years since the discovery of this pathogen, *E. coli* O157:H7 has become increasingly prominent, causing an estimated 20,000 illnesses and 250 deaths each year in the United States alone (6); in 1994, the CDC reported 30 separate outbreaks of this pathogen (CDC surveillance data). Public interest in *E. coli* O157:H7 grew enormously in the wake of a large multistate outbreak in the western United States in early 1993 which resulted in more than 700 illnesses and four deaths (7–10).

Many of the questions that faced the investigators of the original outbreak in 1982 remain unanswered today. Prominent among these is the question of why this pathogen suddenly emerged as a public health problem. Is *E. coli* O157:H7 a completely new pathogen which suddenly appeared in the food supply or is this a pathogen which was present but unrecognized prior to 1982? If *E. coli* O157:H7 infection is truly increasing in incidence, what factors are promoting its emergence and, more importantly, what can be done to stop the spread of this microbe?

In this review we will explore the epidemiology of *E. coli* O157:H7, using it as a model of a new pathogen which has emerged within a sophisticated, highly-developed industry in a "first-world" nation. We will first review the epidemiology of this organism and examine the evidence that supports the paradigm illustrated in figure 1. In the following sections we will explain why we believe *E. coli* O157:H7 is an emerging pathogen, and will explore several hypotheses to explain its emergence as a public health problem. The epidemiology of non-O157 Shiga toxin-producing *E. coli* will not be discussed in this review.

Nomenclature

Because the toxins of *E. coli* O157:H7 are toxic to Vero cell cultures, and are similar to Shiga toxin of *Shigella dysenteriae*, they have become known alternatively as verotoxin 1 and 2 and as Shiga-like toxin I and II; similarly, the strains of *E. coli* that produce these toxins have been known interchangeably as verotoxin-producing *E. coli* or as Shiga-like toxin-producing *E. coli*. There has recently been a proposal to designate the above toxins as *E. coli* Shiga toxin (Stx) types 1 (Stx1) and 2 (Stx2), and to designate the strains of *E. coli* that produce these toxins as Shiga

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Abbreviations: CDC, Centers for Disease Control and Prevention; Stx, *Escherichia coli* Shiga toxin; Stx1, *Escherichia coli* Shiga toxin type 1; *Escherichia coli* Shiga toxin type 2; USDA, United States Department of Agriculture.

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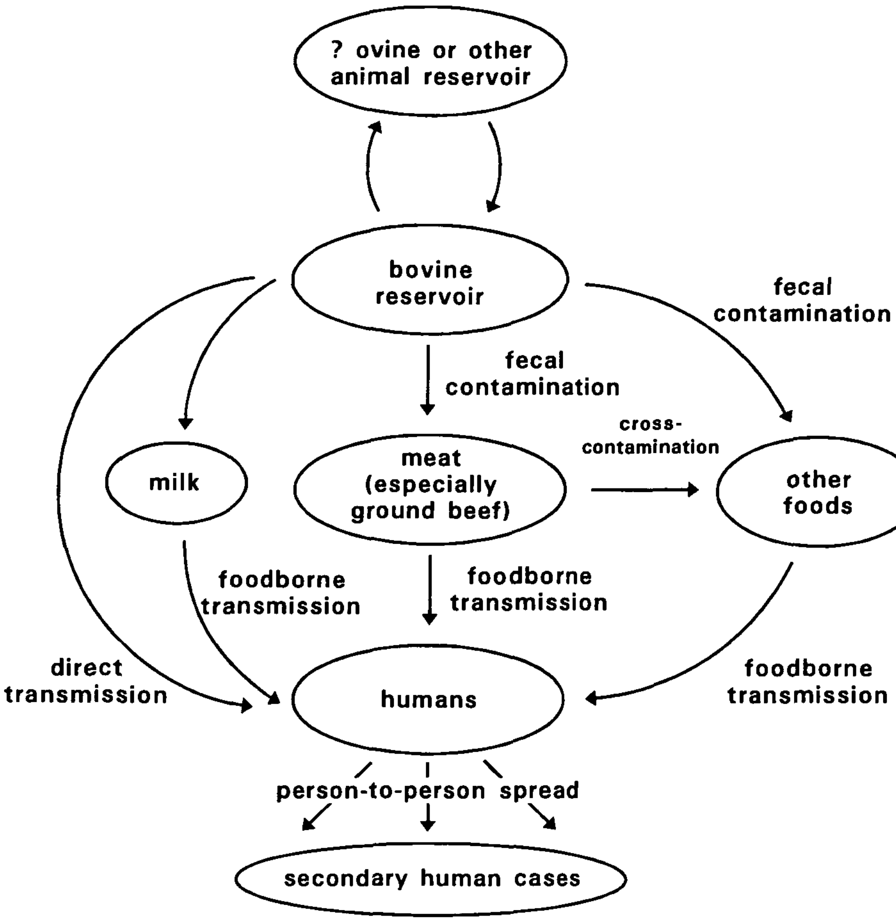

FIGURE 1. Reservoirs and modes of transmission of *Escherichia coli* O157:H7.

toxin-producing *E. coli.* As we believe this proposed nomenclature will become accepted, we will use it in this review.

"Stx-producing *E. coli* O157" is almost synonymous with *E. coli* O157:H7. This is because the only non-H7 serotypes of Shiga toxin-producing *E. coli* O157 are nonmotile and may be *E. coli* O157:H7 strains that have lost their flagellar antigen (5).

EPIDEMIOLOGY OF *E. COLI* O157:H7

E. coli O157:H7 and cattle

A bovine reservoir of *E. coli* O157:H7 has been suspected ever since the first human outbreak was linked with ground beef consumption in 1982. Prior to that time, neither the US Department of Agriculture (USDA) Animal Laboratories nor the Pennsylvania State University Veterinary Research Laboratory had ever detected this serotype in any of its samples (1). Since then, numerous studies in several countries have shown that this organism is present in the gastrointestinal tract of varying percentages of cattle (table 1).

Screening methods used in prevalence studies in cattle. The large differences in the prevalence seen in the studies listed in table 1 are, in part, an artifact of the different methods used to detect *E. coli* O157:H7. Testing for this organism is far from standardized, and the tests in use vary markedly in their sensitivity. Most of the tests involve two distinct steps, screening and confirmation. The classic screening medium for *E. coli* O157:H7 is sorbitol MacConkey agar. This method exploits the fact that *E. coli* O157:H7, unlike 93 percent of other human *E. coli* isolates, does not ferment sorbitol rapidly (2, 11). Overnight growth on MacConkey agar, in which sorbitol has been substituted for lactose, produces clear colonies. Because *E. coli* O157:H7 is not the only non-sorbitol fermenter to grow on this medium, confirmation must be performed by serotyping. Most laboratories place an arbitrary limit on the number of colonies on any given sorbitol MacConkey agar plate that are serotyped.

One approach to increasing the sensitivity of tests for *E. coli* O157:H7 has been the development of more selective screening media. This has the effect of increasing the sensitivity of the overall test since this reduces the number of colonies to be serotyped and increases the amount of stool that can be assayed. The second approach has been the use of selective enrichment media which allows *E. coli* O157:H7 to grow at a faster rate than other non-sorbitol fermenters. Fecal samples are generally grown overnight on selective enrichment broth before being plated on a screening medium such as sorbitol MacConkey agar. Immunomagnetic separation has also been used to improve the sensitivity of these assays (12).

The assertion that the use of different tests can yield different estimates of prevalence has been confirmed by surveys in which more than one assay was used. For example, by using selective enrichment followed by immunomagnetic separation, Chapman et al. (12) were able to detect *E. coli* O157:H7 in 84 (8.2 percent) of 1,024 bovine rectal swabs, whereas direct plating on sorbitol MacConkey agar with cefixime and tellurite and sorbitol MacConkey agar with cefixime and rhamnose detected only 23 (2.2 percent) positives. In another survey, the use of a selective enrichment step increased by almost twofold the isolation of *E. coli* O157:H7 from bovine fecal samples (13). More recently, Sanderson et al. (14) demonstrated that testing larger samples of cattle feces (10 g) is more sensitive than testing smaller samples (fecal swabs), that enrichment broth increases the sensitivity of the assay by a factor of more than 10, and that the use of very selective screening media increases the sensitivity by a factor of 100. Their most sensitive method, selective enrichment on trypticase soy broth with cefixime and vancomycin, followed by culture on sorbitol MacConkey agar with cefixime and tellurite, was almost 200 times more sensitive than direct plating on sorbitol MacConkey agar alone (14).

Absent from table 1 are studies which screened for *E. coli* O157:H7 by using serotyping, Stx detection, or DNA hybridization with probes for Shiga toxin-producing *E. coli* (15–20). Though some of these methods are very useful for detecting Shiga toxin-producing *E. coli* in feces, they are probably insensitive to *E. coli* O157:H7 because of the large number of non-O157:H7 Shiga toxin-producing *E. coli* in cattle. The fact that none of these studies found *E. coli* O157:H7 is likely due to this lack of sensitivity.

Limitations of the cattle data. Although many studies of the prevalence of *E. coli* O157:H7 in cattle have been published, the large variety of screening techniques in use limits our ability to compare them. As might be expected, studies using very sensitive techniques (Chapman et al. (21) and Sanderson et al. (14)) have found the highest prevalences. Nonetheless, examining differences between subgroups within studies has shown some consistent patterns (see below).

Laboratory factors may not be the only cause of the differences seen in these studies; the setting in which the samples are taken (abattoir versus feedlot, for example) may influence the prevalence (22). Regional variations in prevalence were at one time postulated as a possible explanation for the higher incidence of human disease in the northwestern United States compared with the southern United States. However, a study of dairy herds (23) found no significant regional variations in the prevalence of *E. coli* O157:H7. Furthermore, a similar study of feedlot cattle (24) found a slightly higher prevalence in southern herds compared with northern herds. Seasonal variations in prevalence may also influence the results of these studies (25).

All of the studies shown in table 1 have examined the prevalence of *E. coli* O157:H7 in cattle by measuring fecal shedding. It is possible that the principal location of this organism in these animals is not the distal gastrointestinal tract but, rather, the rumen. The fact that this organism is relatively acid-tolerant, which may represent an adaptation to the acid environment of the rumen, and the fact that this organism can be found in other ruminants, such as sheep and deer, supports this hypothesis. If this is the case, then fecal shedding may be a poor measure of prevalence in these animals. This "rumen hypothesis" is discussed in more detail later.

Conclusions from prevalence studies in cattle. Despite the limitations of the prevalence data, three generalizations can be made: 1) *E. coli* O157:H7 can be isolated from the feces of both healthy and ill cattle—estimates of the prevalence in North American and European cattle range from less than 1 percent to almost 10 percent. 2) *E. coli* O157:H7 has a wide geographic distribution in the United States—in one large survey it was found in 63 percent of feedlots (24). 3) *E. coli* O157:H7 is more common in calves than in older cattle.

The last conclusion is supported by one of the earliest surveys of cattle herds, performed in Washington State and Wisconsin, which showed a higher prevalence of this organism in heifers and calves (17 of 604) than in adult cattle (1 of 664) (26). Subsequent studies have consistently shown that young animals have the highest prevalence rates, although the youngest animals show relatively low rates, perhaps reflecting a lack of contact with other animals shedding this organism. The relatively high prevalence in young animals is consistent with the fact that calves, when infected experimentally with this bacterium, shed the organism for a longer period of time than do older cattle (27). A similar pattern has been noted in hu-

TABLE 1. Surveys of the prevalence of *Escherichia coli* O157:H7 in cattle

Study (reference no.)	Site	Prevalence (%)	Numbers of animals*	Type of cattle studied	Screening method†
Surveys of healthy cattle chosen at random					
Clarke et al., 1988 (160)	Ontario, Canada	1.50	3/200	Beef cows at an abattoir	
		0.50	1/200	Dairy cows at an abattoir	
Montenegro et al., 1990 (161)	Germany	0.77	2/259	Healthy bulls and cows at a Berlin slaughterhouse	HC agar or monensin potassium chloride with MUG to isolate *E. coli*, then DNA hybridization to Stx‡ genes
Wells et al., 1991 (26)	Wisconsin	0.37	1/269	Adult cows on farms and at a stockyard	SMAC, or m-TSB followed by SMAC
		2.4	4/168	Heifers and calves in same setting	
Blanco et al. 1993 (162)	Spain	1.79	2/112	Healthy calves	SMAC
Martin et al., 1994 (163)	United States	0	0/304	Calves <10 days old	SMAC-BCIG and m-EC
Hancock et al., 1994 (135)	Washington State	0.28	10/3,570	Dairy cattle	SMAC and TSB-V, then plating on SMAC-MUG
		0.71	10/1,412	Pastured beef cattle	
		0.33	2/600	Feedlot cattle	
US Department of Agriculture (NDHEP§) (164)	United States	0.36	25/6,894	Preweaned calves	m-EC and m-TSB
Zhao et al., 1995 (13) (NDHEP follow-up)	United States (herds previously testing negative)	1.5	6/399	Calves 24 hours old to weaning	dm-TSB-AC followed by SMAC
		4.9	13/263	Calves weaning to 4 months old	
US Department of Agriculture (COFE‖) (24)	United States	1.61	191/11,881	Feedlot cattle	m-EC and m-TSB
Surveys of ill cattle or of cattle on farms or abattoirs implicated in outbreaks					
Chapman et al., 1989 (165)	England	0.97	2/207	Cattle arriving at an implicated abattoir	SMAC (no flagellar antigen typing done)
Wells et al., 1991 (26)	Wisconsin	0	0/169	Adult dairy cows on implicated farms and adjacent farms	SMAC or m-TSB followed by SMAC
		6.4	6/94	Heifers and calves in the same setting	
	Washington State and Oregon	0	0/224	Adult dairy cows on implicated farms	SMAC
		2.1	7/342	Dairy heifers and calves on implicated farms and a packing-house	
Synge and Hopkins,1992 (166)	Scotland	0.40	5/1,247	Ill cattle	SMAC
Chapman et al., 1993 (21)	England	9.52	10/105	Dairy cattle from an implicated farm	Immunomagnetic separation and secondary enrichment (IMSE)

Blanco et al., 1993 (162)	Spain	0.51	1/197	Calves with diarrhea	SMAC
Chapman et al., 1993 (167)	England	3.71	78/2,103	Cattle arriving at an implicated abattoir	CR-SMAC
Synge et al., 1993 (88)	Scotland	1.19	1/84	Dung pats on an implicated farm	SMAC and TC-SMAC
Zhao et al., 1995 (13) (NDHEP follow-up)	United States (herds previously testing positive)	2.9 5.3	5/171 7/132	Calves 24 hours old to weaning Calves weaning to 4 months old	SMAC and dm-TSB-AC followed by SMAC
Sanderson et al., 1995 (14)	United States (herds previously testing positive)	6.8	24/351	Calves 2–8 months old	TSB-CV followed by various plating tech niques

* Number of cattle testing positive for *E. coli* O157:H7 over number of cattle tested.

† Abbreviations for screening methods used: MUG, 4-methylumbelliferyl-β-D-glucuronide; SMAC, direct plating on sorbitol MacConkey agar (11); SMAC-BCIG, direct plating on SMAC with 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide (BCIG) (168); SMAC-MUG, direct plating on SMAC with MUG (169); CR-SMAC, direct plating on SMAC with cefixime and rhamnose (170); TC-SAMC, direct plating on SMAC with cefixime and potassium tellurite (171); HC agar, direct plating on media with tryptone, sorbitol, sodium chloride, bile salts, MUG, and bromocresol purple (172); m-EC, selective enrichment with modified *E. coli* broth with novobiocin (173); m-TSB, selective enrichment with trypticase soy broth (TSB) with bile salts, dipotassium phosphate, and novobiocin (43); TSB-CV, selective enrichment with TSB with cefixime and vancomycin (14); dm-TSB-AC, selective enrichment with TSB with bile salts, dipotassium phosphate, casamino acids, and acriflavin-hydrochloric acid (44); and TSB-V, selective enrichment with TSB with vancomycin (135).

‡ Stx, *E. coli* Shiga toxin.

§ NDHEP, National Dairy Heifer Evaluation Program.

‖ COFE, Cattle on Feed Evaluation.

mans: young children shed *E. coli* O157:H7 longer than do adults (28–30). The longer duration of shedding seen in calves, compared with older cattle, is not unique to *E. coli* O157:H7; *E. coli* strains in general are shed more frequently and in higher numbers in calves compared with adult cattle (31).

Because comparisons of similarly aged dairy and beef cattle using comparable methods have not been published, it is not known if a difference in prevalence exists between these two populations. If significant differences are in fact present, they should be evaluated in light of the different structure of these two sectors of the livestock economy (see below).

Role of E. coli O157:H7 in bovine populations. *E. coli* O157:H7 is generally not pathogenic in adult cattle. Numerous studies have found this organism in the feces of healthy cattle, indicating that it is usually a harmless commensal in these species (see table 1). Nonetheless, other studies have found the organism in the feces of calves with diarrhea, suggesting that it may cause diarrhea in young animals (see table 1). Recently, experimental infection of 17 calves was followed by a transient diarrhea in four, though the cause of the diarrhea, which has a high incidence in such animals under normal circumstances, could not be definitively attributed to the *E. coli* O157:H7 (27). In the same study, all of the 12 adult cattle infected with the organism remained healthy. Both the calves and the adults showed asymptomatic shedding of the organism, though the calves shed it for a longer duration.

Though *E. coli* O157:H7 is probably not pathogenic in cattle, recent studies at Kansas State University indicate that it may be the cause of idiopathic cutaneous and renal glomerular vasculopathy (or "Alabama rot"), a disease very similar to the hemolytic uremic syndrome which is only known to affect greyhounds. Preliminary data indicate that the disease only occurs in animals fed a particularly low grade of raw meat ("4-D" meat, obtained from dead, dying diseased, or disabled cattle), that the organism can be isolated from this meat, and that the dogs shed *E. coli* O157:H7 temporarily after developing the disease (B. Kenwick, Kansas State University, personal communication, 1995).

Other animal reservoirs of E. coli O157:H7. Two groups have surveyed sheep populations using methods sensitive for *E. coli* O157:H7. In Idaho, a survey of a single flock found that the prevalence of fecal shedding varied from 0 percent in November to 31 percent in June (25). In the United Kingdom, a survey of 700 sheep at a slaughterhouse found the organism in the feces of 18 (2.6 percent), a rate lower than that of cattle at the same site (32). Two other surveys have found only non-O157 Stx-producing *E. coli* strains in various farm animals in England (18) and in Germany (17). Neither of these studies used assays sensitive for *E. coli* O157:H7.

In one outbreak of the hemolytic uremic syndrome in northern Italy, illness was linked to contact with chicken coops (33). Although natural infection of chickens with *E. coli* O157:H7 has never been demonstrated, young chicks (1 day old) can be experimentally infected with this organism (34, 35) and will shed it in their feces and the surface of their eggs for up to 11 months (35). The susceptibility of chickens to colonization by *E. coli* O157:H7 drops significantly during the first 3 days of life (36), and can be reduced by feeding birds anaerobic cultures of adult bird fecal bacteria (37).

An ongoing study of *E. coli* O157:H7 in Washington State has begun to shed light on the epizootiology of this organism on farms (38, 39). So far, this serotype has been isolated from two asymptomatic dogs, a deer, and one horse, as well as from environmental sources, including stable flies and drinking water. The two isolates from the deer matched isolates from cattle sharing the same pasture. None of 106 rodent samples were positive.

E. coli O157:H7 in foods

Estimates of the infectious dose of E. coli O157:H7. The low concentrations of *E. coli* O157:H7 found in foods responsible for outbreaks has confirmed what has been suspected from epidemiologic studies—the infectious dose of this organism is small. The hamburger patties implicated in the large multistate outbreak in 1993, for example, had fewer than 700 organisms each before they were cooked, and probably contained considerably fewer by the time of consumption (10). Investigation of an outbreak due to dry-cured salami (40) estimated the infectious dose to be fewer than 50 organisms; some of the cases had probably consumed fewer than five organisms (41). Continued investigations of outbreaks will probably provide our only source of information about factors affecting this infectious dose; human volunteer experiments would be unethical.

Surveys of E. coli O157:H7 in beef products—the importance of using sensitive assays. Because the sorbitol MacConkey agar alone is usually not sensitive enough to detect *E. coli* O157:H7 in such low concentrations, numerous other more sensitive methods have been developed. Table 2 shows some of these methods (reviewed by Padhye and Doyle (42)).

Doyle and Schoeni (43) were the first to develop a sensitive assay for *E. coli* O157:H7 in foods. The results of their trials with artificially inoculated meats indicate their test could detect as few as 1.5 organisms per gram of food. Using their technique, they surveyed retail meats collected from stores in the Madison, Wisconsin, area and found the organism on several types of meat: one (0.7 percent) of 147 ground beef samples, three (1.2 percent) of 250 pork samples, four (1.6 percent) of 257 poultry samples, and four (2.0 percent) of 200 lamb samples tested positive for *E. coli* O157:H7. In the same study, samples collected from around Calgary, Alberta, Canada, and analyzed by the same method showed an even higher prevalence of *E. coli* O157:H7, which was present in five (31 percent) of 17 ground beef samples, one (7 percent) of 14 pork samples, but none of six poultry samples or of five lamb samples.

Since this study, other surveys of retail ground beef have found results similar to those from Madison. Two surveys in Wisconsin, in 1990 and 1991, found that three (2.8 percent) of 107 (44) and one (1.3 percent) of 76 (45) samples were positive. Most probable number determinations in three of these samples estimated that the organism was present at 0.4 to 1.5 organisms per gram (44). Another survey in Manitoba, in 1989, found that four (2.4 percent) of 165 ground beef samples were positive (46).

Not surprisingly, surveys using assays insensitive to low numbers of *E. coli* O157:H7 have failed to find this organism in meat products. A total of 66 retail ground beef samples from Newfoundland (47), 660 samples from Ontario meat processors (48), 255 chicken and sausage samples from the United Kingdom (49), 310 beef products from London, England, retailers (50), and 294 meat and seafood products from around Seattle, Washington (51) all tested negative for *E. coli* O157:H7. Four of these studies (48–51) were designed primarily to detect Shiga toxin-producing *E. coli*, and found non-O157 Shiga toxin-producing *E. coli* to be common. The fifth study (47) used sorbitol MacConkey agar alone, which, as noted above, lacks sensitivity.

The only large-scale survey of beef products using an appropriately sensitive method for *E. coli* O157:H7 detection is currently taking place as part of the USDA's National Microbiological Monitoring Program. This survey makes use of a variation of the "dipstick assay" (52), which reportedly can detect 0.1 to 1.3 organisms per gram (45). During the first year of the *E. coli* O157:H7 testing program, 5,000 samples were tested. Approximately half of these (2,500) came from specimens obtained at processing plants and half (2,500) came from retail stores. In both categories, half of the samples (1,250) were obtained by random sampling of all sites, whereas the other half (1,250) were obtained through targeted sampling of plants or

TABLE 2. Methods for detecting *Escherichia coli* O157:H7 in food

Study (reference no.)	Method*	Reported sensitivity (cells/g)
Szabo et al., 1986 (172)	HC agar	1,000–10,000
Doyle and Schoeni, 1987 (43)	m-TSB Filtering through hydrophobic grid membrane (HGM) Incubation of membranes on nitrocellulose paper Immunoblotting of nitrocellulose paper Confirmation with biochemical, verocytotoxicity, and serologic tests	1.5
Todd et al., 1988 (174)	Mixing with peptone water, maceration by stomaching, filtering to remove food particles Filtering through hyrdophobic grid membrane (HGM) Incubation of membranes on HC agar Immunostain of membrane with HP-labeled m-Ab	10
Szabo et al., 1990 (142)	Same as Todd et al. (174), but with m-TSB	<1
March and Ratnam, 1989 (11)	Latex agglutination	(Very nonspecific)
Okrend et al., 1990 (173)	Selective enrichment on *E. coli* broth, bile salts, novobiocin SMAC Streaking on eosin methylene blue agar and stabbing into phenol red sorbitol agar with MUG Biochemical and serologic confirmation	0.4–0.6
Okrend et al., 1990 (168)	Same as Okrend et al. (173), with SMAC-BCIG (this adds specificity to the selective media)	0.7
Okrend et al., 1990 (176)	m-EC Innoculation onto 3M Petrifilm (3M Corporation, St. Paul, MN) *E. coli* count plates Immunoblotting with O157 antiserum Streaking of positive strains onto SMAC or SMAC-BCIG Serologic testing	0.6
Tison, 1990 (177)	Fluorescein-labeled PAb	
Samadpour et al., 1990 (178)	m-TSB Colony blot hybridization with Stx 1† and Stx2† probes	1.3
Padhye and Doyle, 1992 (44)	dm-TSB-AC Sandwich ELISA‡ of broth with PAb as capture antibody and MAb as detection antibody	0.2–0.9
Kim and Doyle, 1992 (45)	dm-TSB-AC Dipstick immunoassay with PAb as captire antibody and MAb as detection antibody	0.1–1.3
Wright et al., 1994 (77)	Immunomagnetic separation with anti-O157 coated beads Subculture on TC-SMAC	2
Fratamico et al., 1995 (179)	Multiplex polymerase chain reaction with primers for eaeA gene, Stx1 and Stx2, and 60-megadalton plasmid	1,000 organisms

* Abbreviations for methods used: HC agar, direct plating on agar with tryptone, sorbitol, sodium chloride, bile salts, 4-methylumbelliferyl-β-D-glucuronide (MUG), and bromocresol purple (172); m-TSB, selective enrichment with trypticase soy broth (TSB) with bile salts, dipotassium phosphate, and novobiocin (43); HP-labeled m-Ab, horseradish peroxide-labeled monoclonal antibody; SMAC, direct plating on sorbitol MacConkey agar (11); SMAC-BCIG, direct plating on SMAC with 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide (BCIG) (168); m-EC, selective enrichment with modified *E. coli* broth with novobiocin (173); PAb, polyclonal antibody; MAb, monoclonal antibody (also m-Ab); dm-TSB-AC, TSB with bile salts, dipotassium phosphate, casamino acids, and acriflavin-hydrochloric acid (44); and TC-SMAC, SMAC with cefixime and potassium tellurite (171).

‡ Stx1 and Stx2, *E. coli* Shiga toxins 1 and 2.

§ ELISA, enzyme-linked immunosorbent assay.

retailers deemed to be potentially higher risks for contamination. Of the 5,000 tests, only three were positive. It is unclear why such a low prevalence has been found. It is possible that there are regional differences in the prevalence of food contamination, that the prevalence is relatively high in areas such as western Canada where the incidence of human disease is relatively high (53, 54), and that the prevalence is low in areas like the southern United States where few outbreaks have been reported and the reported incidence is low. It is also possible that reductions in rates are related to efforts on the part of the meat industry to reduce the rate of contamination of *E. coli* O157:H7 in ground products.

E. coli O157:H7 in milk. The only other food to be tested systematically for *E. coli* O157:H7 is milk. In a study of 1,021 filters from milk processors (55), it was found that 20 were positive for Shiga toxin-producing *E. coli.* The assay used was insensitive to *E. coli* O157:H7 and, therefore, none was found to be present. A more sensitive assay was used in a second study in which *E. coli* O157:H7 was looked for in raw milk from bulk tanks on farms; it was found to be present in 11 (10 percent) of 115 samples (44).

E. coli O157:H7 in other foods. Unrefrigerated sandwiches (56), potatoes (57), apple cider (58), mayonnaise (59), cantaloupe (60), and lettuce (10) have all been suspected or implicated as vehicles in outbreaks of *E. coli* O157:H7. In such cases involving nonbovine foods, cross contamination by beef or contamination with bovine fecal material has often been suspected (see below).

E. coli O157:H7 in water

Outbreaks involving drinking water. Outbreaks of *E. coli* O157:H7 associated with drinking water have been described. The largest of these occurred in late 1989 in a Missouri town with an unchlorinated water supply (61). This outbreak, which occurred just after severe cold weather had caused two water mains to burst, resulted in 243 cases with four deaths.

Drinking water, which was probably contaminated with bovine feces, was implicated in outbreaks in both Scotland (62) and southern Africa (63). Another outbreak in Japan was caused by well water contaminated from an unknown source (64). In two of these outbreaks, *E. coli* O157 was isolated from the water (63, 64).

Swimming-associated outbreaks. Swimming-associated outbreaks have been reported in Oregon (65), New York (66), Illinois (10), and Wisconsin (M. Proctor, Wisconsin Department of Health and Social Services, personal communication, 1995); illness in these instances was presumably due to swallowing small amounts of water during swimming. In each of these outbreaks, illness was associated with swimming in a specific freshwater swimming area during a specific period of time (in one case, this exposure was limited to a 24-hour period) (65). In none of these outbreaks was the initial source of contamination identified, though there has been speculation, based on anecdotes, that prior to these outbreaks a child with *E. coli* O157:H7 infection may have contaminated the swimming areas (65, 66). In the Oregon and Illinois outbreaks, high levels of *E. coli* contamination were noted in the swimming areas around the time of the outbreaks, though *E. coli* O157:H7 could not be isolated from the water. In another cluster of cases, water in a children's wading pool was identified as a possible vehicle (67).

Transmission of *E. coli* O157:H7 from cattle to humans

Foodborne transmission in outbreaks. Most of our knowledge about the transmission of *E. coli* O157:H7 has come from investigations of outbreaks. Since the first hamburger-related outbreak in 1982, over 80 outbreaks and clusters in the United States have been reported to the CDC, and many others have been described in other countries. Table 3 provides a summary of the vehicles or modes of transmission implicated in outbreaks of *E. coli* O157:H7 in the United States up to and including 1994. Ground beef is the vehicle responsible for the largest portion (58 percent) of foodborne *E. coli* O157:H7 outbreaks.

Attempts to confirm the sources of these outbreaks by testing meat samples have been hampered by the fact that the product has often been completely consumed before the outbreak is recognized and in-

TABLE 3. Outbreaks of *Escherichia coli* O157:H7 reported to the Centers for Disease Control and Prevention (CDC) from 1982 to 1994 inclusive*

Likely vehicle or mode of spread	No. of outbreaks	No. of individuals involved
All foods†	38	1,541
Ground beef†	22	1,137
All beef products and milk†	26	1,278
Drinking water or swimming-associated	3	276
Person-to-person (no food identified)	9	243
Unknown	19	274
All outbreaks	69	2,334

* Source: CDC surveillance data. Note: data from 1982–1992 are incomplete. Outbreaks without clear sources or sites were not tallied by the CDC during that time.

† Some of these outbreaks also involved person-to-person spread.

vestigated. Nonetheless, in at least six ground beef-associated outbreaks, *E. coli* O157:H7 has been isolated from the implicated meat (1, 9, 68–71). In three of these outbreaks (9, 69, 71) molecular subtyping was performed, and this subtyping confirmed that the meat isolate matched the outbreak isolate. In only a single instance has traceback succeeded in identifying the farm from which the infected animal originated. In this case, which occurred in New Jersey, the family of the infected patient had purchased a side of beef from a small producer. Ground beef in the family's freezer contained 500–1,000 CFU/g of *E. coli* O157:H7. The other side of the cow was traced and was found to contain *E. coli* O157:H7 with the same pulsed-field gel electrophoresis pattern but at a lower concentration (100 CFU/g) ((71), Paul Meade, CDC, personal communication, 1994). In another outbreak (69), traceback uncovered six cattle colonized with *E. coli* O157:H7, though the strains differed genetically from the outbreak strain.

It is not surprising that traceback has failed to reveal the outbreak strain in an animal reservoir. Several strains can be found in a single herd of cattle (13, 26, 72), and *E. coli* O157:H7 is shed only intermittently by cattle (13, 73). More importantly, as will be discussed below, traceback is usually hampered by modern production methods in which meat from large numbers of cattle go into any given lot of ground beef.

Even in foodborne outbreaks in which beef is not implicated, contamination with cattle feces is often suspected. In an outbreak linked to apple cider consumption (58) it was found that the apples had been taken from the ground of an orchard next to a cow pasture. Though environmental and bovine fecal cultures were negative in this study, they were obtained 2 months after the outbreak. An outbreak in the United Kingdom was linked to potatoes that had been grown in peat that may have been contaminated with manure (5, 57). A cluster of cases in Maine was linked to vegetables that had been grown in a manured garden (74). An *E. coli* O157:H7 isolate was grown from the soil of this garden which matched an isolate obtained from one of the cases. In some water-associated outbreaks, contamination with bovine feces has been suspected (see above).

Cow's milk has been documented as a vehicle for *E. coli* O157:H7 infection. Two cases of pediatric hemolytic uremic syndrome investigated by the CDC in 1986 were the first to be linked to raw milk consumption (75). The milk in these cases came from two separate farms, both of which had heifers which tested positive for *E. coli* O157:H7. One of 23 raw milk specimens collected from the farms at a later date also tested positive (26). In the same year, an outbreak occurred in Canada involving 43 of 60 children who had visited a dairy farm (76). Infection was significantly associated with consumption of unpasteurized milk at the farm. Fecal testing of 67 cows and calves on the farm yielded at least one positive for *E. coli* O157:H7. Similarly, an investigation of a cluster of cases in the United Kingdom linked to raw milk consumption also found the organism in rectal swabs from heifers on the implicated farm as well as in milk obtained from the positive animals (21, 77). The bovine isolates in this case matched the human isolates in phage type and in Stx production. Pasteurized milk (78) and yogurt (79) have also been implicated in outbreaks. In one case, *E. coli* O157:H7 of the same phage type as the human isolates was recovered from environmental samples taken from the bottling machinery (78).

Foodborne transmission in sporadic E. coli O157: H7. Though the source of *E. coli* O157:H7 in sporadic infections (i.e., cases not associated with a known outbreak) is less clear, existing data show that transmission from bovines plays an important role here as well. Uncontrolled studies of sporadic *E. coli* O157:H7 have noted ground beef (28, 53), rare ground beef (80), and unpasteurized milk (53, 80) as possible vehicles of infection, though a lack of controls in these studies precluded implicating these sources. Six case-control studies of sporadic illness have been performed in the United States and Canada (54, 81–85). The most commonly identified risk factor in these studies was consumption of *undercooked* ground beef (54, 81–83, 85). *E. coli* O157:H7 infection was also associated with consumption of ground beef in a non-commercial setting such as a picnic or "special event" (83), drinking of well water (85), swimming (85), handling animal feces (85), and close contact with a person with diarrhea (82, 85). This latter risk factor may be especially important in children (52). In a New Jersey study (84), the strongest risk factor for *E. coli* O157:H7 was the failure to wash one's hands after handling raw ground beef. No other risk factors were significantly associated with infection when this survey was examined using multivariate analysis. Curiously, a Canadian study found consumption of ground beef in casseroles and consumption of hamburger cooked at home to be protective (odds ratio = 0.5) (83). The authors of this study suggested that this effect may have been due to more thorough cooking of the ground beef in these foods as compared with other foods containing ground beef.

Studies comparing *E. coli* O157:H7 strains from cattle with those from sporadic human infections have shown varying amounts of overlap. In a study in Washington State, 22 bovine and 50 sporadic human isolates were compared by plasmid typing, bacteriophage lambda restriction fragment length polymorphism, and Stx production (72). Among the 77 isolates, 43 separate strains were identified, of which only three were found in both humans and cattle. Of the human isolates, 10 percent (5 of 50) were strains also found in cattle. Of the bovine isolates, 27 percent (6 of 22) were strains also found in humans. Conflicting results were reported in a British study in which 96 cattle isolates and 63 sporadic human isolates were compared by phage typing, plasmid typing, and Stx production (87). In these 159 isolates, only 41 different strains were identified. Of the human isolates, 94 percent (59 of 63) were strains also found in the cattle, although only 26 percent (10 of 39) of cattle isolates were found in humans. The smaller degree of overlap seen in the Washington State study was probably due to the more discriminatory typing methods used and may also reflect that meat consumed in that state does not necessarily originate from there.

Direct transmission from bovines to humans. Two instances of apparent direct transmission of *E. coli* O157:H7 from bovines to humans have been documented. In Canada, in 1992, a 13-month-old child became ill with *E. coli* O157:H7 after having direct, prolonged contact with calves on his family's farm (73). Two calves on the farm tested positive for *E. coli* O157:H7 of the same phage type and Stx type as the child. It was noted that the family did not drink unpasteurized milk and had not consumed undercooked ground beef. A similar case occurred in Scotland in which a 15-month-old child living adjacent to a farm became ill with *E. coli* O157:H7 (88). One of 84 dung samples from the farm yielded *E. coli* O157:H7 of the same phage type and the same unusual plasmid profile as the human isolate. It was speculated that the family dog, which frequently roamed the farm, may have acted as a vector in the case. Though direct transmission from cattle to humans may occur as purported in these cases, such transmission appears to be rare.

Person-to-person transmission

Secondary transmission in outbreaks. Secondary transmission after point-source outbreaks of *E. coli* O157:H7 is common. An example is the 1988 outbreak in Minnesota linked to undercooked hamburger patties served at a junior high school (89). A total of 32 cases were reported, including a 12-year-old student whose mother ran a day-care center. One week after this student's illness, two of the children in the day-care center developed hemorrhagic colitis and were culture positive for *E. coli* O157:H7. Numerous other instances of secondary transmission during outbreaks have been reported (30, 40, 56, 58, 65, 69, 78, 90–95), including the large outbreak in 1993 in the western United States in which 48 of the original 501 cases were classified as secondary (9). Such secondary cases usually represent a relatively small portion (less than 10 percent) of the total number of cases in any outbreak and occur within 1–2 weeks of the primary cases. Sustained person-person transmission after outbreaks has never been reported except in the setting of day-care centers, a special situation that will be discussed later.

Because asymptomatic cases can occur in outbreaks, there has been concern that persons with such infections could unwittingly spread their infection to others. The existence of such asymptomatic cases during outbreaks has been well documented (30, 56, 67, 90–92, 95, 96). Asymptomatic infections have also been demonstrated in family members and other close contacts of persons with hemolytic uremic syndrome or symptomatic *E. coli* O157:H7 (29, 93, 97).

The fact that secondary transmission can occur through an asymptomatic case was demonstrated in a restaurant-associated outbreak in Scotland in 1990 (93). Five of the 16 reported cases in this outbreak had not eaten at the implicated restaurant and were presumed to have acquired the infection from someone who had. One of these secondary cases was an 8-year-old girl whose father had eaten at the restaurant but who had not developed diarrhea. Both the girl and her father tested positive for *E. coli* O157:H7 of the same phage type and Stx type as the outbreak isolate. As with other secondary cases, cases due to transmission from asymptomatic individuals are few in number but highlight the importance of preventing foodborne and other common source outbreaks of *E. coli* O157:H7.

Duration of shedding. Long-term asymptomatic carriage (greater than 1 year) of *E. coli* O157:H7 has never been demonstrated, as with enteric pathogens such as *Salmonella typhi*. Stool cultures of over 500 asymptomatic unexposed individuals tested since 1980 have failed to show any *E. coli* O157:H7 (5), a sample size which is large enough to exclude a true prevalence of more than 0.7 percent (with $p \leq 0.05$). Though long-term carriers may not exist, infected individuals, especially children, can shed the organism for several weeks after the onset of their illness (29, 30, 98). A study of transmission of *E. coli* O157:H7 in day-care centers in Minnesota (30) found the mean duration of shedding to be 17 days for the 24 children studied. One child, who had been treated with amoxicillin, excreted the organism for 62 days. A second study of 53 children infected with *E. coli* O157:H7 (98) found the median duration of shedding to be 13 days, with one child shedding the organism for 124 days. Children often shed this organism intermittently (30, 98) and without symptoms (98, 99).

These studies may overestimate the average duration of shedding because 1) they involve only children, who shed the organism longer than do adults and 2) because they do not include data from children who had very short durations of shedding or who were asymptomatic and not diagnosed as having *E. coli* O157:H7. Another method to examine the duration of shedding is to look at the isolation rate of *E. coli* O157:H7 in persons who develop diarrhea after a known exposure to the organism or to look at this isolation rate after diarrhea-associated hemolytic uremic syndrome. These studies show that the rate of *E. coli* O157:H7 isolation from stool falls to 50 percent by 6–8 days after the onset of symptoms (1, 28, 100–103). In one study of post-diarrhea hemolytic uremic syndrome (102), the isolation rate was 100 percent for those stools collected within 2 days of the onset of diarrhea. This dropped to 92 percent at 3–6 days and 33 percent after 6 days.

Person-to-person transmission in day-care centers. Person-to-person transmission is of special importance in day-care centers, where it may be the primary mode of transmission. In this context, data are very comparable to those obtained for *Shigella*, another enteric pathogen with a low infectious dose, which is known to be an important cause of day-care center outbreaks (104). In 1988, after initiating surveillance for *E. coli* O157:H7, the Minnesota Department of Health found evidence of continuing person-to-person transmission in all nine day-care facilities in which children with the infection had been identified (30). In six of these facilities the state recommended that all of the children be excluded from the facility until two consecutive stool cultures tested negative. This action resulted in the cessation of transmission at each facility. In another day-care center outbreak, transmission was stopped by cohorting ill children until they were culture negative (S. Shah, Colorado Department of Public Health, personal communication, 1995), an approach which has also proven successful in the control of shigellosis (105). Person-to-person spread was implicated as the primary mode of transmission in two other day-care center outbreaks (95, 100), and probably played an important role in an outbreak involving institutions for mentally retarded persons (92).

Recent trends in *E. coli* O157:H7 outbreaks

Outbreaks involving acidic foods, such as mayonnaise (59) and apple cider (58), have underscored the unusual acid tolerance of this organism. Acidic foods (defined by the US Food and Drug Administration's Retail Food Sanitation Code as those with a pH of less than 4.6) are generally considered to be at low risk for transmission of pathogenic bacteria. But *E. coli* O157:H7, under certain circumstances, can survive a pH as low as 2.0 (106, 107) and can persist for up to several weeks when inoculated into apple cider (58, 107, 108) or mayonnaise (109–111). This acid tolerance may have also contributed to a 1994 outbreak involving dry cured salami (40, 41). This product, considered ready to eat, is not cooked during processing; rather, producers rely on the low pH attained by the product during fermentation to kill pathogenic microbes. Why *E. coli* O157:H7, presumably an enteric bacteria, should be so acid tolerant is unclear and has led to speculation about its reservoir (see below).

Leaf lettuce has been implicated in two separate outbreaks in 1995 (CDC surveillance data). Although it isn't known if the lettuce in these cases was directly contaminated on the farm or if it was cross-contaminated from a different source after harvest, it is known that *E. coli* O157:H7 can grow on lettuce at temperatures as low as 12°C (112).

"EMERGENCE" OF *E. COLI* O157:H7

While investigating the first recognized *E. coli* O157:H7 outbreaks in 1982, CDC personnel were impressed by the severity of the illness they were witnessing. Never before had the CDC investigated an outbreak of hemorrhagic diarrhea of unknown etiology (113). The subjective impression that they were dealing with a genuinely novel pathogen spurred the exhaustive investigation that led to the discovery of *E. coli* O157:H7 as a cause of epidemic hemorrhagic colitis. Starting with this first recognized outbreak, the number of outbreaks reported to the CDC has been increasing steadily since 1982, from fewer than a half dozen per year in the 1980s to 17 in 1993 to 30 in 1994 (figure 2). In terms of its recognition and impact on public health policy, *E. coli* O157:H7 is clearly a "new" emerging pathogen, with reported outbreaks forming a traditional, steeply inclined epidemic curve.

The factors underlying this emergence are less clear. Is *E. coli* O157:H7 a "new" pathogen at a molecular level (i.e., did one strain of *E. coli* suddenly acquire new virulence genes, resulting in occurrence of a totally new disease), is it new to cattle or a cattle reservoir, or is it's "newness" related solely to occurrence or recognition in humans? If it is not a totally new organism, what factors have contributed to its recent appearance in human populations and its associated recognition as a major public health problem?

Is *E. coli* O157:H7 a new pathogen?

At the time of the first *E. coli* O157:H7 outbreaks, public health laboratories in the United States, the

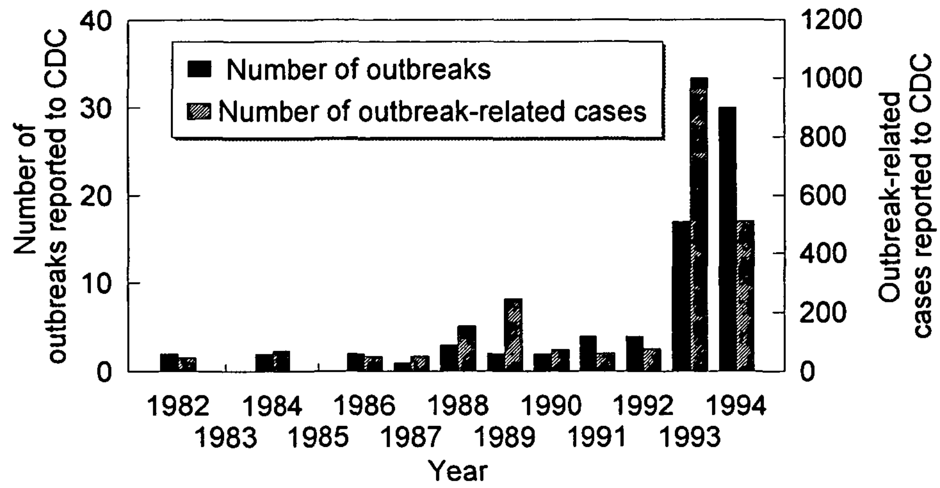

FIGURE 2. Number of outbreaks and outbreak-related cases of *Escherichia coli* O157:H7 reported to the Centers for Disease Control and Prevention (CDC). (Source: CDC surveillance data).

United Kingdom, and Canada reviewed the serotypes of the isolates that had been sent to them over the previous decade. Since 1973, the CDC had serotyped over 3,000 specimens of *E. coli* that had been sent to their laboratory; only one of these, a 1975 isolate from a Californian woman with grossly bloody diarrhea, was of serotype O157:H7 (1). Similarly, the United Kingdom's Public Health Laboratory reported that it had found this serotype in one of 15,000 *E. coli* isolates studied between 1978 and 1982 (114). The Laboratory Centre for Disease Control in Canada, on the other hand, found this serotype in six of 2,000 isolates sent to them during the same years (115). Two of the patients from which these isolates were obtained had hemorrhagic colitis; clinical information about the other four patients was not available.

Serologic evidence of *E. coli* O157 infection has also been found in stored serum samples. European investigators studying serum from Dutch hemolytic uremic syndrome patients, found antibodies to the O157 antigen in 14 of the 33 patients who presented between 1974 and 1981 (116). The proportion of samples testing positive showed a statistically significant increase with the time (chi-square test for trend, $p < 0.05$), an indication the portion of hemolytic uremic syndrome due to *E. coli* O157:H7 may have been increasing.

As noted above, reported outbreaks of *E. coli* O157:H7 disease have shown a dramatic increase since the first outbreaks were recognized in the early 1980s. The incidence of sporadic *E. coli* O157:H7 during this time is more difficult to determine because of misdiagnosis and underreporting. Although 33 states currently require reporting of *E. coli* O157:H7 (10), many of these only began tracking this disease following a recommendation by the Council of State and Territorial Epidemiologists in June 1993. Additionally, many laboratories have only recently begun testing for *E. coli* O157:H7 on a regular basis, spurred by this recommendation and the recommendations of local and state health departments; from 1991 to 1994, the number of laboratories routinely testing stools for *E. coli* O157:H7 increased from 4 percent to 29 percent (10). This increase in testing has had an impact on the number of reported cases: in the summer of 1994, for example, an investigation of a striking increase in the number of reported cases in New Jersey found no evidence of an outbreak, but suggested instead that the increase in cases was a result of increased testing (71).

Because the diagnosis and reporting of *E. coli* O157:H7 has not been consistent over the last decade, it is conceivable that the observed increase in incidence is artifactual. One indirect gauge of the incidence of *E. coli* O157:H7 is the incidence of hemolytic uremic syndrome in children. This may be a more reliable indicator of trends in *E. coli* O157:H7 incidence because the severity of the illness makes it less prone to ascertainment bias.

Most hemolytic uremic syndrome in North America is caused by *E. coli* O157:H7 (5). A Canadian study of pediatric hemolytic uremic syndrome, for example, found that 76 percent of all children with the disease tested positive for *E. coli* O157:H7 (117). In half of these children, stool cultures were obtained more than 6 days after the onset of their diarrhea, a factor which likely reduced the chance of finding the organism. A similar study in Washington State found a rate of 63 percent in all patients, and a rate of 96 percent in patients whose stools were cultured within 6 days of the onset of their symptoms (102). In this study, all of the 13 patients tested within 2 days of onset tested positive for *E. coli* O157:H7.

Three retrospective studies in the United States have examined trends in the incidence of hemolytic uremic syndrome during the 1970s and 1980s. A 10-year study in Minnesota (1979–1988) (118) and a 15-year study in Washington State (1971–1986) (119) both documented more than twofold increases in the incidence of hemolytic uremic syndrome over the study periods. Both studies looked for and failed to find any evidence of referral or ascertainment bias to explain the observed increases. On the other hand, a 20-year retrospective study of cases in Utah (1971–1990) failed to find any increase in incidence (120). This study also examined the clinical presentation of the children and noted no changes, a fact that the authors cited as evidence that the proportion of hemolytic uremic syndrome due to *E. coli* O157:H7 was not changing.

Possible emergence of *E. coli* O157:H7 in cattle populations

To our knowledge, there has been only one documented case of *E. coli* O157:H7 infection in cattle prior to 1982. This was found to be the predominant serotype in one of 13 calves with coli bacillosis studied in Argentina in 1977 (121). On the other hand, when the serotyping records of both the USDA Animal Laboratories in Ames, Iowa, and the Pennsylvania State University Veterinary Research Laboratory were reviewed in 1982, neither laboratory had isolated *E. coli* O157:H7 from any animal sources (2). It should be noted that serotyping is an inefficient method of isolating *E. coli* O157:H7 (see above) and may not have been sensitive enough to detect this organism had it been present. Since the sensitivity of serotyping is unknown and the numbers of isolates serotyped by these two labs was not published, it is not possible to determine the likelihood that *E. coli* O157:H7 would have been detected had it been as prevalent in cattle then as it is now.

Molecular origin of *E. coli* O157:H7

Analyses based on multilocus enzyme electrophoresis (122, 123), as well as examination of the variable (3′) portion of the eae gene (124, 125), have shown a remarkable similarity between *E. coli* O157:H7 isolates from diverse geographic sources and a remarkable divergence of O157:H7 from other Shiga toxin-producing *E. coli* serotypes. Furthermore, analysis of multilocus enzyme electrophoresis data has shown that the evolution of O157:H7 probably began on the order of 5 million years ago when it and *E. coli* O55:H7 (a serotype of enteropathogenic *E. coli*) descended from a common ancestor. Following this divergence, O157:H7 lost its ability to ferment sorbitol and later it's ability to produce β-D-glucuronidase. During this evolution it also acquired Stx2, then Stx1, and possibly other virulence factors. Unfortunately, one cannot determine, using available data, when this organism acquired sufficient virulence to cause hemolytic uremic syndrome in humans, nor can one determine when it emerged in animal populations (126).

Summary

There would appear to be little argument that the large outbreaks of *E. coli* O157:H7 which have occurred since the early 1980s represent a distinct, new phenomenon. The number of reported cases have increased dramatically, starting from zero in 1981; however, it is also clear that this increase in reported cases is in part an artifact of improved surveillance and reporting. Available data suggest that *E. coli* O157:H7 infections were present prior to 1982, although numbers appear to have been small. At a molecular level, the organism shows evidence of clonal origin, but there is not the striking clonality, with virtually identical pulsed-field gel electrophoresis and ribotyping patterns, which has been seen in situations such as the emergence of *Vibrio cholerae* O139 Bengal in the Indian subcontinent in 1992 or the introduction of *V. cholerae* O1 into naïve populations in South America in 1991 (127–129). Findings are more consistent with the image of an organism which arose from a common ancestor, but which has had time to become distributed geographically and to show some evidence of genetic divergence. While this is an "emerging" infection, at least in terms of its distribution and public recognition, it is unlikely that it will be possible to identify the "first" O157:H7 case or to track the clonal spread of the organism through cattle or human populations.

FACTORS THAT MAY HAVE LED TO THE EMERGENCE/RECOGNITION OF *E. COLI* O157:H7

As noted above, the occurrence of large outbreaks and the widespread distribution of cases do appear to be a new phenomenon. To account for the emergence of this organism in human populations, we can consider three broad hypotheses: 1) conditions for the spread of *E. coli* O157:H7 from animals to humans have always existed, but this organism has only recently emerged in animal populations; 2) *E. coli* O157:H7 has always been widespread in animal populations, but slaughter and meat processing practices have changed in such a way as to promote contamination of meat with this organism; or 3) *E. coli* O157:H7 has always been present in the meat supply, but consumer practices have changed such that contaminated meat now leads to human infection. These hypotheses are not mutually exclusive: it is certainly possible that changes at multiple levels of the food chain have been responsible for the emergence of this organism.

Changes in the livestock industry

There is little evidence to support or refute the hypothesis that the emergence of *E. coli* O157:H7 in human populations is the result of its emergence in cattle herds. Geographic spread of this organism has never been demonstrated as it has for emerging *Salmonella* serotypes, such as serotypes Hadar, Agona, or Wien (130). Similarly, there have been relatively few studies examining factors that might influence the prevalence of *E. coli* O157:H7 in cattle. Nevertheless, because an understanding of the ecology of this organism within its reservoir is important to understanding its epidemiology, we feel it important to discuss some of the factors that have been proposed as influences on this ecology as well as the data that have been published so far.

Structural changes in the livestock industry. There has been speculation that structural changes in the livestock industry may have created conditions favorable for the emergence of *E. coli* O157:H7. Today, cattle can often pass through several geographic locations before going to slaughter, giving them more opportunities to come in contact with other infected animals. Beef cattle are generally bred by small cow/calf producers, raised on roughage until they are 400–600 pounds (181.4–272.2 kg) (roughly 6–9 months), and sold to growing operations (also known as "backgrounders" or "stocker operations"). The cattle continue growing on a diet of roughage, gaining another 200–300 pounds (90.7–136.1 kg), before being sold to a feedlot. There they are fed to an end weight of 1,050–1,250 pounds (476.3–566.0 kg) using enriched grains, and subsequently sold to a slaughterhouse.

The dairy cattle industry is structured differently. Animals are generally born on site at the dairy farms, used for breeding and milk production for 3–5 years, and then sold directly to a slaughterhouse when milk output slows. They are older than beef cattle at the time of slaughter and produce meat of poorer quality that is often used in ground beef.

In the beef cattle industry, the upper level (the feedlots) has become more concentrated over the last 20 years, whereas the lower levels (the cow/calf producers and the grower operations) have continued to remain relatively dispersed (131). The number of feedlots, for example, decreased from 121,000 in 1970 to 43,000 in 1988 (132). This decrease was entirely the result of the reduction in number of the smallest feedlots (those with a onetime capacity of less than 1,000 head of cattle). The number of feedlots with over 1,000 head steadily increased during this time. The largest lots, those with 16,000 head or more, increased their market share from 5.7 percent in 1962 to 40.4 percent in 1989. While this was occurring, there was a simultaneous shift in the location of feedlots from the upper midwestern states (South Dakota, Minnesota, Iowa, and Illinois), where they were in close proximity to the meatpacking industry, to the lower midwestern states (Nebraska, Kansas, Colorado, Texas, and Oklahoma) where the warmer climate resulted in lower costs. The number of fed cattle in the Upper Midwest decreased from 4,782,000 in 1960 (roughly half of the US total) to 3,490,000 in 1989, whereas the number of fed cattle in the Lower Midwest increased from 3,385,000 to 17,140,000 during the same period (133).

The US dairy industry has also increased in concentration over the last two decades. The number of commercial dairy farms decreased from 600,000 in 1955 to 160,000 in 1989, while the number of farms with milk cows decreased from 2,800,000 to 205,000. During the same time period, there was a regional shift in milk production from the Northeast and Upper Midwest to the West and Southwest (especially California) (134).

Whether these changes in the livestock industry played any role in the emergence of *E. coli* O157:H7 is uncertain. It is possible that the increased concentration of cattle on these lots has led to an increase in the prevalence of this pathogen in animals going to slaughter. Similarly, it is conceivable that increased movement of cattle throughout the states or the change in location of feedlots to more southern climates could have influenced this emergence.

Changes in cattle management practices. Management practices on individual farms also may have created conditions favorable for the emergence of *E. coli* O157:H7. Two case-control studies, one in Washington State (135) and one on farms across the United States (23), have examined the correlation of management practices with the presence of *E. coli* O157:H7. The first of these studies (135) found only one factor that correlated positively and significantly with infection: the use of computerized feeding. Such feeding systems result in more frequent and more controlled feeding (136). It is not clear, however, how such a system would promote the spread of *E. coli* O157:H7. The second study (23), which looked only at calves, found that the grouping of these calves prior to weaning increased the risk of infection by *E. coli* O157:H7. Curiously, both studies found that feeding of whole cottonseed was apparently protective against infection. The significance of this is unclear.

Numerous other cattle management practices were examined in these studies but did not correlate with colonization. The use of "ionophores" (carboxylin polyether ionophore antibiotics) to increase feed efficiency, a practice which began in the mid 1970s, is one example of these. Ionophores are known to alter the microbial flora of the rumen by inhibiting gram-positive bacteria and thereby promoting gram-negative bacteria. In doing so, they alter the fermentation process such that protein degradation is decreased, methane production is decreased, and propionic acid production is increased (137). Because the first *E. coli* O157:H7 outbreaks occurred shortly after the introduction of ionophores, there has been speculation that physiologic changes brought about in the rumen by these agents might select for this organism. However, there has been no experimental evidence to date to support this (136). One case-control study which compared 64 farms found no association between ionophore use and the presence of *E. coli* O157:H7-positive cattle (23).

It has also been suggested that the increased use of manure slurries to fertilize pastures may have played a role in the emergence of *E. coli* O157:H7. This organism can not only survive, but can grow in manure slurries for at least several weeks (138). Although the use of manure slurries has increased markedly since the 1970s, two case-control studies have failed to find a significant association between the use of manure slurries and the presence of *E. coli* O157:H7 (23, 135).

The decline in naturally-occurring brucellosis is a third factor which has been suggested to account for the emergence of *E. coli* O157:H7 (113). Because the somatic O157 antigen cross reacts with antigens of *Brucella* species (139, 140), it is possible that lower brucellosis immunity could promote the emergence of *E. coli* O157:H7. In fact, in the United States, the incidence of human *E. coli* O157:H7 is highest in the Northwest (5, 141), where the incidence of bovine brucellosis is relatively low. One case-control study which examined the relation between brucellosis vaccination and the presence of *E. coli* O157:H7 found no association (23). Studies comparing the presence of brucellosis antibodies with the presence of *E. coli* O157:H7 in the stool of individual cattle have yielded conflicting results (142, 143).

The possible role of rumen physiology in E. coli O157:H7 shedding. In order to understand the ecology of *E. coli* O157:H7 in cattle, and to develop effective control measures for this pathogen, it is important to remember that cattle are ruminants. Ruminants include cows, sheep, goats, deer, and other herbivores that possess large four-chamber stomachs that act as fermentation vats to break down nutrients, especially cellulose, which these animals would otherwise be unable to digest. The environment in this key portion of the gastrointestinal tract may influence fecal shedding of *E. coli* O157:H7 and other pathogens.

The rumen, the largest of the four chambers of a ruminant's stomach, is the site of most of the fermentation. When the animal feeds, rumen bacteria and protozoa break down the complex polysaccharides into two important groups of products, methane and carbon dioxide, which are eliminated through eructation (burping), and short-chain fatty acids ("volatile fatty acids"), which are absorbed by the animal and used as carbon and energy sources. The concentration of volatile fatty acids (usually 50–200 mM) and the pH of the rumen (usually 5.0–7.5) depend upon the nutritional status of the ruminant: the concentration of volatile fatty acids will be high and pH low in the rumen of a well-fed animal, whereas the inverse will be true in a starving animal. A similar physiology is seen in the cecum of the animal, where a lesser amount of cellulose fermentation occurs.

Since volatile fatty acids inhibit the growth of enteric organisms (144–146), one would expect that the presence or absence of enteric bacteria in the rumen would depend upon the nutritional status of that animal, i.e., that rumen fluid from a well-fed animal would contain few enteric organisms, whereas that from a poorly fed animal might even support their growth. A series of experiments in the 1960s by Brownlie, Grau, and others in Australia confirms this (146–149). In well-fed animals, *Salmonella*, when introduced artificially, was rapidly eliminated from the rumen fluid and could not be detected in the feces. If the feed intake of the animal was then reduced to one-half to one-third of what it would normally eat, the numbers of *E. coli* in the fluid would rise; *Salmonella*, when introduced artificially, would persist for 2–3 days but would not be shed in detectable numbers in the feces. When food was cut off completely for 2–3 days, *E. coli* counts would climb even higher; *Salmonella* would grow in the fluid and would be shed in the feces in detectable numbers. When feeding was subsequently restarted, numbers of both species would fall again, but only after a growth spurt in the initial 6 hours, during which numbers of both organisms climbed to their highest levels measured during these experiments (146, 149).

The implication of these observations on modern abattoir practice was highlighted in experiments by the same authors, in which rumen fluid and feces from animals awaiting slaughter were sampled for *Salmonella* (148). By the time most animals are slaughtered, they have been starved for a variable period of time. The reasons for this are twofold: first, animals are generally not fed in transit from the feedlot to the slaughterhouse, and second, animals with relatively empty rumina (which can account for one-sixth of a ruminant's total weight) are easier to eviscerate. In this study, *Salmonella* prevalence in the rumen increased with the amount of time elapsed since the animal's last feeding. The highest prevalence was in animals which had been fed once after their arrival at the plant. The prevalence of *Salmonella* in the feces was also examined in this study and did not show a statistically significant correlation with the time since the previous feed. A similar study two decades later corroborated these findings (144), and a study in Thailand found that animals at slaughter had a higher prevalence of Shiga toxin-producing *E. coli* (80 percent) than animals on farms (60 percent) (15).

In a study using a sheep model, investigators in Idaho showed that diet has a clear effect on fecal shedding of *E. coli* O157:H7 (138). Two types of feed were given during the experiment, alfalfa pellets, a concentrate feed such as is given in feedlots, and sagebrush-bunchgrass, which is high in fiber and, like other high-fiber feeds, results in lower levels of ruminal volatile fatty acids than do concentrate feeds. As might have been predicted, the authors found that by withholding feed they could induce fecal shedding of *E. coli* O157:H7 in animals artificially inoculated with this organism. When the inoculated lambs were fed alfalfa pellets, they and their non-dosed pen mates shed the organism only intermittently. But, when they were subsequently released to feed on a sagebrush-bunchgrass range, all the animals, including the non-dosed sheep, shed the organism but then cleared it, apparently completely. Even when feed was withheld, the animals could no longer be induced to shed the organism. These findings confirm the effect of diet on *E. coli* O157:H7 shedding and suggest that the ruminal environment, especially the concentration of volatile fatty acids, may play an important role.

It has been hypothesized that rumen physiology may also explain the higher prevalence of *E. coli* O157:H7 in calves compared with older animals. Just after birth, the anatomy and diet of the animal support little fermentation, leading to low production of volatile fatty acids in the rumen. In this "preruminant" stage, *E. coli* and other enteric organisms are normal inhabitants of the ruminal fluid. When these animals are weaned, fermentation increases, and along with it production of volatile fatty acids, which one might expect to decrease the shedding of *E. coli* O157:H7. Unfortunately, this hypothesis is not supported by prevalence studies, in which weaned calves shed *E. coli* O157:H7 more frequently than preweaned calves (13, 23, 135).

Another finding that sheds doubt on the rumen as the main reservoir of *E. coli* O157:H7 is the finding that, although this serotype is unusually acid tolerant, it is no more tolerant of ruminal conditions than other serotypes of *E. coli* and is, in fact, less tolerant than a strain isolated originally from rumen fluid (145).

Despite these shortfalls, these observations taken together have implications for epizootiologic studies and possibly for the control of this pathogen in ruminants: 1) Since fecal shedding of *E. coli* O157:H7 depends upon diet, the nutritional status of individual animals should be taken into account whenever prevalence studies are performed. Temporarily withholding feed may be a method to increase the likelihood of detecting this organism in colonized animals. 2) If the rumen is in fact the reservoir of *E. coli* O157:H7 *or* if it is the source of *E. coli* O157:H7 that contaminates meat in slaughterhouses, then surveys of fecal shedding by animals may have little relevance to public health. 3) Since animals going to slaughter are generally in a temporary state of starvation, and it is known that starvation causes *E. coli* and *Salmonella* to proliferate in the rumen, research is needed to determine if practical feeding strategies or other means to elevate the concentration of volatile fatty acids in the rumen can be developed in order to reduce the numbers of these pathogens in animals at the time of slaughter.

Changes in food processing and the food industry

Mass production of ground beef. Methods currently used to produce ground beef make it possible for meat from dozens or even hundreds of cattle to go into any given hamburger patty. To produce ground beef, large commercial meat packers may purchase raw meat from several different sources, both domestic and foreign. Examination of the records of one such producer, for example, revealed that several lots were produced each day. Into each of these lots, which ranged in size from 2 tons (1.8 metric tons) to almost 30 tons (27.2 metric tons), went boneless boxed beef from two to 11 different sources located in two to four different states. Some of these sources were purveyors, who had in turn purchased carcasses from several different slaughterhouses. Complicating the matter was that all of the lots from any given day had been produced sequentially in the same meat grinder without cleaning the machinery between lots. Such a continuous throughput process makes it impossible to identify the discreet start and end points of production lots, thereby making it possible for meat or contaminants from one lot to be mixed with those of another. The "coarse ground beef" produced by this producer was then packaged in 80 pound (36.3 kg) "chubs" (tubes) and sent to grocers. Upon receiving this meat, the grocers reground it along with "table trimmings" (usually fat trimmed from more expensive cuts) and with meat cuts that had been on their shelves for more than 2 days. Therefore, when the product was finally purchased by the consumer and made into hamburgers, it was nearly impossible to say which cattle (or even how many) went into the patties. Another example of the difficulty in determining the origin of contaminated meat is provided by the large outbreak in the western United States in 1993. Meat that could have contaminated the implicated lots of ground beef came from three different suppliers who had received meat from the United States, New Zealand, and Canada. Even when the investigation focussed only on the single most likely supplier, traceback led to 443 individual cattle, which had come from six different states through five different slaughterhouses (150).

How much ground beef could a single infected animal contaminate?. Given that investigations such as these have not led to the identification of the infected cattle, one could question whether it is possible for a single infected animal to infect a large lot of ground beef. At a hypothetical level, we can estimate the impact of a single contaminated carcass by using the following assumptions: average weight of a dressed cattle carcass in the United States: 688 pounds (312.1 kg) (151); weight of carcass after removing bone and fat: 66 percent × dressed carcass weight (152); degree of contamination with *E. coli* O157:H7: 750 CFU/g on one side and 100 CFU/g on other side (from the New Jersey example cited above); number of organisms necessary to cause human illness: 700 CFU (the number of CFU in the hamburger patties implicated in the 1993 multistate outbreak prior to cooking) (10); weight of uncooked hamburger patty: 1/4 pound (113.4 g). With the above numbers, assuming that the bacteria is distributed evenly throughout the carcass and that one half of the carcass is used to make ground beef, there would be sufficient numbers of bacteria on the carcass to contaminate 8 tons (7.3 metric tons) of ground beef.

The above calculations are hypothetical, and require assumptions which are unlikely to be met in "real life" slaughter and processing operations. Nonetheless, these calculations underscore the potential for widespread distribution of the organism (in numbers sufficient to cause human illness) from a single contaminated source. The Food Safety and Inspection Service and the USDA are aware of three instances in which ground beef manufacturers have detected *E. coli* O157:H7 in their product through in-house microbiologic testing. Each of these involved large-scale producers whose machines ground 4,000–12,000 pounds (1645.6–5443.1 kg) per hour continuously for 20-hours each day, shutting down only for a 4-hour cleaning each night. The serial cultures obtained from the production lines in each case showed that the pathogen appeared suddenly in the product and subsequently disappeared after 1–2 hours, despite the fact that the machinery had not been cleaned in the interim. In two of these cases, data on total coliform counts were also available for review: These counts rose dramatically just prior to the appearance of the *E. coli* O157:H7 and fell back to normal when the *E. coli* O157:H7 disappeared. In one of the cases, testing of the raw materials had failed to show the pathogen, presumably because it had not been evenly distributed throughout the unground meat. In none of these cases could the source of the contaminated raw product be identified because of the large number of suppliers and because of the almost fluid nature of the product.

Increased size of beef processors. Concentration and vertical integration in the meat industry has increased the size of meat producers in the last 20 years. This has, in turn, led to an increase in the size of ground beef lots potentially increasing the volume of contaminated meat and the possibility of outbreaks. Given that only a single infected carcass can contaminate a large lot of ground beef, it is possible that, whereas in the past an infected animal would produce only a small number of cases, such an animal could now cause a large, widespread outbreak.

In fact, the market share, measured in heads of cattle slaughtered, of the four largest meatpacking firms increased from 22 percent in 1977 to 32 percent in 1982 to 54 percent in 1987. The boxed beef industry was even more concentrated in 1987, with 80 percent of the market share going to the four largest firms. Such an increase in concentration is remarkable in the food industry (132), a fact which prompted a congressional hearing in 1990 (153). Nonetheless, the increased concentration in meatpacking cannot by itself account for the emergence of *E. coli* O157:H7; this industry was equally concentrated in 1918 when the Federal Trade Commission chose the "Big Five" meatpackers (the "Beef Trust") to be the target of one of its first investigations (132). Also, there is some evidence that microbial contamination is lower in meat from slaughterhouses that process larger numbers of cattle (154).

Changes in consumer preferences

Although certain consumer practices may promote human *E. coli* O157:H7 infection, there is little evidence that changes in consumer practices played any role in the emergence of this organism.

Consumer practices involving ground beef may explain why this food has been the most commonly implicated vehicle in foodborne outbreaks. Ground beef is unlike most other raw meats in that microbial contaminants, which are normally concentrated on the surface of meat are, instead, located throughout the product as a consequence of the grinding. Therefore, when hamburger is eaten rare or medium rare, as is the preference of 23–25 percent of the US population (155, 156), the meat inside the hamburger still contains viable organisms. Because the infectious dose of *E. coli* O157:H7 is exceedingly small, even very low levels of contamination are sufficient to result in infection.

There has been no increase in ground beef consumption in the United States over the last 20 years. On the contrary, data from the American Meat Institute indicate that per capita disappearance (an indicator of consumption) has decreased from a peak of 30.5 pounds (13.8 kg) in 1976 to 26.6 pounds (12.1 kg) in 1993 (157).

On the other hand, the portion of ground beef eaten outside the home probably increased during this time. Although we are unaware of any surveys that have measured this directly, household spending on "meals and snacks" (most of which was eaten in restaurants or hotels) has been increasing at a faster rate than spending on food for "off premise use" (most of which was purchased in retail stores) (158). The largest increases in spending have come in the "fast food" sector. Between 1967 and 1982, spending in restaurants and cafeterias increased 21 percent, whereas spending in "fast food places" increased 224 percent (159). Such a trend towards consumption in public settings may have the effect of increasing the likelihood that microbial contamination will lead to recognizable outbreaks.

CONCLUSIONS

Changes in such elements as society and environment have clearly been identified as factors that have led to the emergence of certain pathogens. Increased urbanization and the "sexual revolution" of the 1960s and 1970s, for example, created conditions ideal for the spread of human immunodeficiency virus. The return of forests to city suburbs, the migration of city dwellers to these suburbs, and the simultaneous increase in deer populations probably all contributed to the emergence of Lyme disease.

On the other hand, the determinants responsible for the emergence of *E. coli* O157:H7 remain elusive. It is unclear even where to look in the food chain to find these determinants. As noted in the preceding sections, the livestock and beef industries have changed dramatically during the past several decades. While no one change can be singled out as the "major" contributing factor to emergence of this pathogen, it is possible that these changes, alone and together, have created a setting in which this organism has been able to spread more readily through and into animal and human populations. In particular, big may not always be better: Consolidation of the industry, widespread movement of cattle, and increased use of large production lots for products such as hamburger may all have played a role in the process—and may provide a setting in which other "new" pathogens can rapidly move into human populations. At the same time, it is possible that this organism would have emerged as a major pathogen regardless of these changes, based on its ability to move into ecologic niches in cattle and its virulence and low infectious dose in humans. It should also be recognized that consolidation can be a positive force, permitting efficient design and implementation of *E. coli* O157:H7 control programs.

There is often an impression that emerging diseases occur "elsewhere," in developing countries where sanitation and medical resources are limited. *E. coli* O157:H7 serves as a model of a pathogen which has appeared in a highly sophisticated industry in a "first world" nation. As the above discussion points out, we still do not know what triggered its appearance. This should provide a cautionary note to those of us working in public health: the need for surveillance in conditions which may foster emergence of new and potentially even more dangerous pathogens.

REFERENCES

1. Riley LW, Remis RS, Helgerson SD, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med 1983;308:681–5.
2. Wells JG, Davis BR, Wachsmuth IK, et al. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J Clin Microbiol 1983;18: 512–20.
3. Karmali MA, Steele BT, Petric M, et al. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet 1983;1:619–20.
4. Karmali MA, Petric M, Lim C, et al. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J Infect Dis 1985;151: 775–82.
5. Griffin PM. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In: Blaser MJ, Smith PD, Ravdin JI, et al., eds. Infections of the gastrointestinal tract. New York, NY: Raven Press, 1995:739–61.
6. Consensus conference statement: *Escherichia coli* O157-H7 infections—an emerging national health crisis, July 11–13, 1994. Gastroenterology 1995;108:1923–34.
7. Preliminary report: foodborne outbreak of *Escherichia coli* O157:H7 infections from hamburgers—western United States, 1993. MMWR Morb Mortal Wkly Rep 1993;42: 85–6.
8. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers—western United States, 1992–1993. MMWR Morb Mortal Wkly Rep 1993;42: 258–63.

9. Bell BP, Goldoft M, Griffin PM, et al. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: the Washington experience. JAMA 1994;272:1349–53.
10. Griffin PM. Symposium on new and emerging pathogens: *Escherichia coli* O157:H7—beyond the burger. In: Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, California, September 17–20, 1995. Washington,, DC: American Society for Microbiology, 1995:355.
11. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J Clin Microbiol 1986;23:869–72.
12. Chapman PA, Wright DJ, Siddons CA. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. J Med Microbiol 1994;40:424–7.
13. Zhao T, Doyle MP, Shere J, et al. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. Appl Environ Microbiol 1995;61:1290–3.
14. Sanderson MW, Gay JM, Hancock DD, et al. Sensitivity of bacteriologic culture for detection of *Escherichia coli* O157:H7 in bovine feces. J Clin Microbiol 1995;33: 2616–19.
15. Suthienkul O, Brown JE, Seriwatana J, et al. Shiga-like-toxin-producing *Escherichia coli* in retail meats and cattle in Thailand. Appl Environ Microbiol 1990;56:1135–9.
16. Wilson JB, McEwen SA, Clarke RC, et al. Distribution and characteristics of verocytotoxigenic *Escherichia coli* isolated from Ontario dairy cattle. Epidemiol Infect 1992;108: 423–39.
17. Beutin L, Geier D, Steinruck H, et al. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J Clin Microbiol 1993;31:2483–8.
18. Wray C, McLaren IM, Carroll PJ. *Escherichia coli* isolated from farm animals in England and Wales between 1986 and 1991. Vet Rec 1993;133:439–42.
19. Tokhi AM, Peiris JSM, Scotland SM, et al. A longitudinal study of Vero cytotoxin producing *Escherichia coli* in cattle calves in Sri Lanka. Epidemiol Infect 1993;110:197–208.
20. Willshaw GA, Cheasty T, Jiggle B, et al. Vero cytotoxin-producing *Escherichia coli* in a herd of dairy cattle. (Letter). Vet Rec 1993;132:96.
21. Chapman PA, Wright DJ, Higgins R. Untreated milk as a source of verotoxigenic *E. coli* O157. (Letter). Vet Rec 1993;133:171–2.
22. Whipp SC, Rasmussen MA, Cray WC Jr. Animals as a source of *Escherichia coli* pathogenic for human beings. J Am Vet Med Assoc 1994;204:1168–75.
23. Garber LP, Wells SJ, Hancock DD, et al. Risk factors for fecal shedding of *Escherichia coli* O157:H7 in dairy calves. J Am Vet Med Assoc 1995;207:46–9.
24. *Escherichia coli* O157:H7 shedding by feedlot cattle: National Animal Health Monitoring System, COFE. Washington, DC: United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, 1995.
25. Kudva IT, Hatfield PG, Hovde CJ. *Escherichia coli* O157:H7 in microbial flora of sheep. J Clin Microbiol 1996;34:431–3.
26. Wells JG, Shipman LD, Greene KD, et al. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J Clin Microbiol 1991;29:985–9.
27. Cray WC Jr, Moon HW. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. Appl Environ Microbiol 1995;61:1586–90.
28. Pai CH, Gordon R, Sims HV, et al. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157: H7: clinical, epidemiologic, and bacteriologic features. Ann Intern Med 1984;101:738–42.
29. Pai CH, Ahmed N, Lior H, et al. Epidemiology of sporadic diarrhea due to verocytotoxin-producing *Escherichia coli*: a two-year prospective study. J Infect Dis 1988;157:1054–7.
30. Belongia EA, Osterholm MT, Soler JT, et al. Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. JAMA 1993;269:883–8.
31. Howe K, Linton AH. A longitudinal study of *Escherichia coli* in cows and calves with special reference to the distribution of O-antigen types and antibiotic resistance. J Appl Bacteriol 1976;40:331–40.
32. Chapman PA, Siddons CA, Harkin MA. Sheep as a potential source of verocytotoxin-producing *Escherichia coli* O157. (Letter). Vet Rec 1996;138:23–4.
33. Tozzi AE, Niccolini A, Caprioli A, et al. A community outbreak of haemolytic-uraemic syndrome in children occurring in a large area of northern Italy over a period of several months. Epidemiol Infect 1994;113:209–19.
34. Beery JT, Doyle MP, Schoeni JL. Colonization of chicken cecae by *Escherichia coli* associated with hemorrhagic colitis. Appl Environ Microbiol 1985;49:310–15.
35. Schoeni JL, Doyle MP. Variable colonization of chickens perorally inoculated with *Escherichia coli* O157:H7 and subsequent contamination of eggs. Appl Environ Microbiol 1994;60:2958–62.
36. Stavric S, Buchanan B, Gleeson TM. Intestinal colonization of young chicks with *Escherichia coli* O157:H7 and other verotoxin-producing serotypes. J Appl Bacteriol 1993;74: 557–63.
37. Stavric S, Buchanan B, Gleeson TM. Competitive exclusion of *Escherichia coli* O157:H7 from chicks with anaerobic cultures of faecal microflora. Lett Appl Microbiology 1992; 14:191–3.
38. Rice DH, Hancock DD, Besser TE. Verotoxigenic *E. coli* O157 colonisation of wild deer and range cattle. (Letter). Vet Rec 1995;137:524.
39. Hancock DD, Besser TE, Rice DH. Non-bovine sources of *Escherichia coli* O157:H7. In: Abstracts of the Proceedings of the Conference of Research Workers in Animal Diseases, Chicago, Illinois, November 13–14, 1995. (Abstract no. 66).
40. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami—Washington and California, 1994. MMWR Morb Mortal Wkly Rep 1995;44:157–60.
41. Tilden J, Young AM, McNamara MC, et al. New routes of transmission of an emerging pathogen: investigation of an outbreak of *E. coli* O157:H7 associated with dry cured salami. In: Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, California, September 17–20, 1995. Washington, DC: American Society for Microbiology, 1955:314.
42. Padhye NV, Doyle MP. *Escherichia coli* O157:H7: epidemiology, pathogenesis, and methods for detection in food. J Food Protect 1992;55:555–65.
43. Doyle MP, Schoeni JL. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl Environ Microbiol 1987;53:2394–6.
44. Padhye NV, Doyle MP. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* O157:H7 in food. Appl Environ Microbiol 1991;57:2693–8.
45. Kim MS, Doyle MP. Dipstick immunoassay to detect enterohemorrhagic *Escherichia coli* O157:H7 in retail ground beef. Appl Environ Microbiol 1992;58:1764–7.
46. Sekla L, Milley D, Stackiw W, et al. Verotoxin-producing *Escherichia coli* in ground beef in Manitoba. Can Dis Wkly Rep 1990;16:103–5.
47. Ratnam S, March SB. Sporadic occurrence of hemorrhagic colitis associated with *Escherichia coli* O157:H7 in Newfoundland. Can Med Assoc J 1986;134:43–5, 49.
48. Read SC, Gyles CL, Clarke RC, et al. Prevalence of verocytotoxigenic *Escherichia coli* in ground beef, pork, and chicken in southwestern Ontario. Epidemiol Infect 1990;105: 11–20.
49. Smith HR, Cheasty T, Roberts D, et al. Examination of retail chickens and sausages in Britain for vero cytotoxin-

producing *Escherichia coli*. Appl Environ Microbiol 1991; 57:2091–3.
50. Willshaw GA, Smith HR, Roberts D, et al. Examination of raw beef products for the presence of Vero cytotoxin producing *Escherichia coli*, particularly those of serogroup O157. J Appl Bacteriol 1993;75:420–6.
51. Samadpour M, Ongerth JE, Liston J, et al. Occurrence of Shiga-like toxin-producing *Escherichia coli* in retail fresh seafood, beef, lamb, pork, and poultry from grocery stores in Seattle, Washington. Appl Environ Microbiol 1994;60: 1038–40.
52. Johnson JL, Rose BE, Sharar AK, et al. Methods used for detection and recovery of *Escherichia coli* O157:H7 associated with a food-borne disease outbreak. J Food Protect 1995;58:597–603.
53. Waters JR, Sharp JC, Dev VJ. Infection caused by *Escherichia coli* O157:H7 in Alberta, Canada, and in Scotland: a five-year review, 1987-1991. Clin Infect Dis 1994;19: 834–43.
54. Bryant HE, Athar MA, Pai CH. Risk factors for *Escherichia coli* O157:H7 infection in an urban community. J Infect Dis 1989;160:858–64.
55. Clarke RC, McEwen SA, Gannon VP, et al. Isolation of verocytotoxin-producing *Escherichia coli* from milk filters in south-western Ontario. Epidemiol Infect 1989;102: 253–60.
56. Carter AO, Borczyk AA, Carlson JA, et al. A severe outbreak of *Escherichia coli* O157:H7—associated hemorrhagic colitis in a nursing home. N Engl J Med 1987;317:1496–500.
57. Morgan GM, Newman C, Palmer SR, et al. First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O 157.H7 in the UK. Epidemiol Infect 1988;101:83–91.
58. Besser RE, Lett SM, Weber JT, et al. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. JAMA 1993;269: 2217–20.
59. Keene WE, Mcanulty JM, Williams LP, et al. A two-restaurant outbreak of *Escherichia coli* O157:H7 enteritis associated with consumption of mayonnaise. In: Abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, Louisiana, October 17–20, 1993. Washington, DC: American Society for Microbiology, 1993:354.
60. Yet another outbreak of hemorrhagic colitis—Corvallis. CD Summary 1993;42:1–2. (Published by: Center for Disease Prevention and Epidemiology, Oregon Health Division, Department of Human Resources, Portland, OR).
61. Swerdlow DL, Woodruff BA, Brady RC, et al. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann Intern Med 1992;117: 812–19.
62. Dev VJ, Main M, Gould I. Waterborne outbreak of *Escherichia coli* O157. (Letter). Lancet 1991;337:1412.
63. Isaacson M, Canter PH, Effler P, et al. Haemorrhagic colitis epidemic in Africa. (Letter). Lancet 1993;341:961.
64. Akashi S, Joh K, Tsuji A, et al. A severe outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7 in Japan. Eur J Pediatr 1994;153:650–5.
65. Keene WE, McAnulty JM, Hoesly FC, et al. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. N Engl J Med 1994;331:579–84.
66. Ackman D, Birkhefad G, Root T, et al. Swimming-associated hemorrhagic colitis due to *Escherichia coli* O157:H7 infection. In: Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, California, September 17–20, 1995. Washington, DC: American Society for Microbiology, 1955:299.
67. Brewster DH, Brown MI, Robertson D, et al. An outbreak of *Escherichia coli* O157 associated with a children's paddling pool. Epidemiol Infect 1994;112:441–7.
68. Hockin J, Lior H, Mueller L, et al. An outbreak of *E. coli* O157:H7 diarrhea in a nursing home—Alberta. Can Dis Wkly Rep 1987;13:206.
69. Ostroff SM, Griffin PM, Tauxe RV, et al. A statewide outbreak of *Escherichia coli* O157:H7 infections in Washington State. Am J Epidemiol 1990;132:239–47.
70. *Escherichia coli* O157:H7 outbreak linked to home-cooked hamburger—California, July 1993. MMWR Morb Mortal Wkly Rep 1994;43:213–16.
71. Enhanced detection of sporadic *Escherichia coli* O157:H7 infections—New Jersey, July 1994. MMWR Morb Mortal Wkly Rep 1995;44:417–18.
72. Paros M, Tarr PI, Kim H, et al. A comparison of human and bovine *Escherichia coli* O157:H7 isolates by toxin genotype, plasmid profile, and bacteriophage lambda-restriction fragment length polymorphism profile. J Infect Dis 1993;168: 1300–3.
73. Renwick SA, Wilson JB, Clarke RC, et al. Evidence of direct transmission of *Escherichia coli* O157:H7 infection between calves and a human. (Letter). J Infect Dis 1993;168:792–3.
74. Cieslak PR, Barrett TJ, Griffin PM, et al. *Escherichia coli* O157:H7 infection from a manured garden. (Letter). Lancet 1993;342:367.
75. Martin ML, Shipman LD, Wells JG, et al. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome. (Letter). Lancet 1986;2:1043.
76. Borczyk AA, Karmali MA, Lior H, et al. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. (Letter). Lancet 1987;1:98.
77. Wright DJ, Chapman PA, Siddons CA. Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. Epidemiol Infect 1994;113: 31–9.
78. Upton P, Coia JE. Outbreak of *Escherichia coli* O157 infection associated with pasteurised milk supply. (Letter). Lancet 1994;344:1015.
79. Morgan D, Newman CP, Hutchinson DN, et al. Verotoxin producing *Escherichia coli* O 157 infections associated with the consumption of yoghurt. Epidemiol Infect 1993;111: 181–7.
80. Ostroff SM, Kobayashi JM, Lewis JH. Infections with *Escherichia coli* O157:H7 in Washington State: the first year of statewide disease surveillance. JAMA 1989;262:355–9.
81. MacDonald KL, O'Leary MJ, Cohen ML, et al. *Escherichia coli* O157:H7, an emerging gastrointestinal pathogen: results of a one-year, prospective, population-based study. JAMA 1988;259:3567–70.
82. Rowe PC, Orrbine E, Lior H, et al. Diarrhoea in close contacts as a risk factor for childhood haemolytic uraemic syndrome: the CPKDRC co-investigators. Epidemiol Infect 1993;110:9–16.
83. Le Saux N, Spika JS, Friesen B, et al. Ground beef consumption in noncommercial settings is a risk factor for sporadic *Escherichia coli* O157:H7 infection in Canada. (Letter). J Infect Dis 1993;167:500–2.
84. Mead P, Finelli L, Spitalny K, et al. Risk factors for sporadic infection with *Escherichia coli* O157:H7. In: Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, California, September 17–20, 1995. Washington, DC: American Society for Microbiology, 1995:314.
85. Ries A, Griffin P, Greene K, et al. Sources of sporadic *Escherichia coli* O157:H7 infection in the United States: a nationwide case-control study. Abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, Louisiana, 1993.
86. Nationwide microbiologic baseline studies. Washington, DC: United States Department of Agriculture, Food Safety and Inspection Service, Science and Technology, 1995.
87. Chapman PA, Siddons CA. A comparison of strains of

Escherichia coli O157 from humans and cattle in Sheffield, United Kingdom. (Letter). J Infect Dis 1994;170:251-3.
88. Synge BA, Hopkins GF, Reilly WJ, et al. Possible link between cattle and *E. coli* O157 infection in a human. (Letter). Vet Rec 1993;133:507.
89. Belongia EA, MacDonald KL, Parham GL, et al. An outbreak of *Escherichia coli* O157:H7 colitis associated with consumption of precooked meat patties. J Infect Dis 1991; 164:338-43.
90. Ryan CA, Tauxe RV, Hosek GW, et al. *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical, epidemiological, and pathological findings. J Infect Dis 1986;154:631-8.
91. Salmon RL, Farrell ID, Hutchison JGP, et al. A christening party outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O 157.H7. Epidemiol Infect 1989;103:249-54.
92. Pavia AT, Nichols CR, Green DP, et al. Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. J Pediatr 1990;116: 544-51.
93. Marsh J, MacLeod AF, Hanson MF, et al. A restaurant-associated outbreak of *E. coli* O157 infection. J Public Health Med 1992;14:78-83.
94. Kohli HS, Chaudhuri AK, Todd WT, et al. A severe outbreak of *E. coli* O157 in two psychogeriatric wards. J Public Health Med 1994;16:11-15.
95. Reida P, Wolff M, Pohls HW, et al. An outbreak due to enterohaemorrhagic *Escherichia coli* O157:H7 in a children day care centre characterized by person-to-person transmission and environmental contamination. Int J Med Microbiol Virol Parasitol Infect Dis 1994;281:534-43.
96. Smith HR, Rowe B, Gross RJ, et al. Haemorrhagic colitis and Vero-cytotoxin-producing *Escherichia coli* in England and Wales. Lancet 1987;1:1062-5.
97. Caprioli A, Luzzi I, Rosmini F, et al. Hemolytic-uremic syndrome and Vero cytotoxin-producing *Escherichia coli* infection in Italy: the HUS Italian Study Group. J Infect Dis 1992;166:154-8.
98. Karch H, Russmann H, Schmidt H, et al. Long-term shedding and clonal turnover of enterohemorrhagic *Escherichia coli* O157 in diarrheal diseases. J Clin Microbiol 1995;33: 1602-5.
99. Orr P, Lorencz B, Brown R, et al. An outbreak of diarrhea due to verotoxin-producing *Escherichia coli* in the Canadian Northwest Territories. Scand J Infect Dis 1994;26:675-84.
100. Spika JS, Parsons JE, Nordenberg D, et al. Hemolytic uremic syndrome and diarrhea associated with *Escherichia coli* O157:H7 in a day care center. J Pediatr 1986;109:287-91.
101. Milford DV, Taylor CM, Guttridge B, et al. Haemolytic uraemic syndromes in the British Isles 1985-8: association with Verocytotoxin producing *Escherichia coli*. Part 1: Clinical and epidemiological aspects. Arch Dis Child 1990;65: 716-21.
102. Tarr PI, Neill MA, Clausen CR, et al. *Escherichia coli* O157:H7 and the hemolytic uremic syndrome: importance of early cultures in establishing the etiology. J Infect Dis 1990; 162:553-6.
103. Bitzan M, Ludwig K, Klemt M, et al. The role of *Escherichia coli* O 157 infections in the classical (enteropathic) haemolytic uraemic syndrome: results of a Central European, multicentre study. Epidemiol Infect 1993;110:183-96.
104. Weissman JB, Schmerler A, Weiler P, et al. The role of preschool children in day-care centers in the spread of shigellosis in urban communities. J Pediatr 1974;84:797-802.
105. Hoffman RE, Shillam PJ. The use of hygiene, cohorting, and antimicrobial therapy to control an outbreak of shigellosis. Am J Dis Child 1990;144:219-21.
106. Conner DE, Kotrola JS. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. Appl Environ Microbiol 1995;61:382-5.
107. Miller LG, Kaspar CW. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. J Food Protect 1994;57: 460-4.
108. Zhao T, Doyle MP, Besser RE. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. Appl Environ Microbiol 1993;59:2526-30.
109. Zhao T, Doyle MP. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in commercial mayonnaise. J Food Protect 1994;57:780-3.
110. Weagant SD, Bryant JL, Bark DH. Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. J Food Protect 1994; 57:629-31.
111. Raghubeer ER, Ke JS, Campbell ML, et al. Fate of *Escherichia coli* O157:H7 and other coliforms in commercial mayonnaise and refrigerated salad dressing. J Food Protect 1995; 58:13-8.
112. Abdul-Raouf UM, Beuchat LR, Ammar MS. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. Appl Environ Microbiol 1993;59:1999-2006.
113. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev 1991;13:60-98.
114. Day NP, Scotland SM, Cheasty T, et al. *Escherichia coli* O157:H7 associated with human infections in the United Kingdom. (Letter). Lancet 1983;1:825.
115. Johnson WM, Lior H, Bezanson GS. Cytotoxic *Escherichia coli* O157:H7 associated with haemorrhagic colitis in Canada. (Letter). Lancet 1983;1:76.
116. Chart H, Rowe B, v d Kar N, et al. Serological identification of *Escherichia coli* O157 as cause of haemolytic uraemic syndrome in Netherlands. (Letter). Lancet 1991;337:437.
117. Rowe PC, Orrbine E, Lior H, et al. A prospective study of exposure to verotoxin-producing *Escherichia coli* among Canadian children with haemolytic uraemic syndrome: the CPKDRC co-investigators. Epidemiol Infect 1993;110:1-7.
118. Martin DL, MacDonald KL, White KE, et al. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. N Engl J Med 1990;323:1161-7.
119. Tarr PI, Neill MA, Allen J, et al. The increasing incidence of the hemolytic-uremic syndrome in King County, Washington: lack of evidence for ascertainment bias. Am J Epidemiol 1989;129:582-6.
120. Siegler RL, Pavia AT, Christofferson RD, et al. A 20-year population-based study of postdiarrheal hemolytic uremic syndrome in Utah. Pediatrics 1994;94:35-40.
121. Orskov F, Orskov I, Villar JA. Cattle as reservoir of verotoxin-producing *Escherichia coli* O157:H7. (Letter). Lancet 1987;2:276.
122. Whittam TS, Wachsmuth IK, Wilson RA. Genetic evidence of clonal descent of *Escherichia coli* O157:H7 associated with hemorrhagic colitis and hemolytic uremic syndrome. J Infect Dis 1988;157:1124-33.
123. Whittam TS, Wolfe ML, Wachsmuth IK, et al. Clonal relationships among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. Infect Immun 1993;61: 1619-29.
124. Willshaw GA, Scotland SM, Smith HR, et al. Hybridization of strains of *Escherichia coli* O157 with probes derived from the eaeA gene of enteropathogenic *E. coli* and the eaeA homolog from a Vero cytotoxin-producing strain of *E. coli* O157. J Clin Microbiol 1994;32:897-902.
125. Louie M, de Azevedo J, Clarke R, et al. Sequence heterogeneity of the eae gene and detection of verotoxin-producing *Escherichia coli* using serotype-specific primers. Epidemiol Infect 1994;112:449-61.
126. Whittam TS. Genetic population structure and pathogenicity in enteric bacteria. In: Baumberg S, Young JPW, Saunders SR, et al., eds. Population genetics of bacteria: Symposium of the Society for General Microbiology. Cambridge, England: Cambridge University Press, 1995:217-45.
127. Morris JG, the Cholera Laboratory Task Force. *Vibrio chol-*

erae O139 Bengal. In: Wachsmuth IK, Blake PA, Olsvik O, eds. *Vibrio cholerae* and cholera: molecular to global perspectives. Washington, DC: ASM Press, 1994:95–102.
128. Wachsmuth IK, Evins GM, Fields PI, et al. The molecular epidemiology of cholera in Latin America. J Infect Dis 1993;167:621–6.
129. Popovic T, Bopp C, Olsvik O, et al. Epidemiologic application of a standardized ribotyping scheme for *Vibrio cholerae* O1. J Clin Microbiol 1993;31:2474–82.
130. D'Aoust JY. *Salmonella*. In: Doyle MP, ed. Foodborne bacterial pathogens. New York, NY: Marcel Dekker, 1989: 327–446.
131. Krause KR. The beef cow-calf industry, 1964–87: location and size. Washington, DC: United States Department of Agriculture, Agricultural Research Service, 1992. (Agricultural economic report no. 659).
132. Johnson DG, Connor JM, Josling T, et al. Concentration issues in the US beef subsector. In: Competitive issues in the beef sector: can beef compete in the 1990s? Minneapolis, MN: University of Minnesota, 1990:65–96.
133. Krause KR. Cattle feeding, 1962–89. Washington, DC: United States Department of Agriculture, Agricultural Research Service, 1991. (Agricultural economic report no. 642).
134. Fallert RF, Blayney DP, Miller JJ. Dairy—background for 1990 farm legislation. Washington, DC: United States Department of Agriculture, 1990.
135. Hancock DD, Besser TE, Kinsel ML, et al. The prevalence of *Escherichia coli* O157.H7 in dairy and beef cattle in Washington State. Epidemiol Infect 1994;113:199–207.
136. *Escherichia coli* O157:H7; issues and ramifications. Washington, DC: United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, 1994.
137. van Nevel CJ. Modification of rumen fermentation by the use of additives. In: Jouany JP, ed. Rumen microbial metabolism and ruminant digestion. Paris, France: Institue National de la Recherche Agronomique, 1991:263–80.
138. Kudva IT, Hatfield PG, Hovde CJ. Effect of diet on the shedding of *Escherichia coli* O157:H7 in a sheep model. Appl Environ Microbiol 1995;61:1363–70.
139. Stuart FA, Corbel MJ. Identification of a serological cross-reaction between *Brucella abortus* and *Escherichia coli* O:157. Vet Rec 1982;110:202–3.
140. Perry MB, Bundle DR. Antigenic relationships of the lipopolysaccharides of *Escherichia hermannii* strains with those of *Escherichia coli* O157:H7, *Brucella melitensis*, and *Brucella abortus*. Infect Immun 1990;58:1391–5.
141. Slutsker L, Ries A, Maloney K, et al. Clinical features of *Escherichia coli* O157:H7 infection: how wise is conventional wisdom? In: Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, California, September 17–20, 1995. Washington, DC: American Society for Microbiology, 1995:314.
142. Szabo R, Todd E, MacKenzie J, et al. Increased sensitivity of the rapid hydrophobic grid membrane filter enzyme-labeled antibody procedure for *Escherichia coli* O157 detection in foods and bovine feces. Appl Environ Microbiol 1990;56: 3546–9.
143. Johnson RP, Boag L, Anderson S, et al. *Brucella abortus* serology in cattle naturally infected with *Escherichia coli* O157.H7. Vet Rec 1994;135:382–3.
144. Mattila T, Frost AJ, O'Boyle D. The growth of *Salmonella* in rumen fluid from cattle at slaughter. Epidemiol Infect 1988; 101:337–45.
145. Rasmussen MA, Cray WC Jr, Casey TA, et al. Rumen contents as a reservoir of enterohemorrhagic *Escherichia coli*. FEMS Microbiol Lett 1993;114:79–84.
146. Brownlie LE, Grau FH. Effect of food intake and growth and survival of salmonellas and *Escherichia coli* in the bovine rumen. J Gen Microbiol 1967;46:125–34.
147. Grau FH, Brownlie LE. Occurrence of salmonellas in the bovine rumen. Aust Vet J 1965;41:321–3.
148. Grau FH, Brownlie LE, Roberts EA. Effect of some preslaughter treatments on the *Salmonella* population in the bovine rumen and faeces. J Appl Bacteriol 1968;31:157–63.
149. Grau FH, Brownlie LE, Smith MG. Effects of food intake on numbers of *Salmonellae* and *Escherichia coli* in rumen and faeces of sheep. J Appl Bacteriol 1969;32:112–17.
150. Tuttle J, Gomez T. EPI-AID trip report: Multistate outbreak of *Escherichia coli* O157:H7 infections—meat traceback. Atlanta, GA: Centers for Disease Control and Prevention, 1993. (Epi 93–33).
151. Red meats yearbook—1994. Washington, DC: United States Department of Agriculture, Economic Research Service, 1994. (Statistical bulletin no. 885).
152. Duewer LA, Krause KR, Nelson KE. US poultry and red meat consumption, prices, spreads, and margins. Washington, DC: United States Department of Agriculture, 1993. (Statistical bulletin no. 684).
153. Hearing on economic concentration in the meatpacking industry. United States Senate Committee on Agriculture, Nutrition, and Forestry, Subcommittee on Nutrition and Investigations, 1990. Washington, DC: US GPO, 1990. (Stock no. S/N 552–070–11346–1).
154. Hogue AT, Dreesen DW, Green SS, et al. Bacteria on beef briskets and ground beef: correlation with slaughter volume and antemortem condemnation. J Food Protect 1993;56: 110–19.
155. Klontz KC, Timbo B, Fein S, et al. Prevalence of selected food consumption and preparation behaviors associated with increased risks of food-borne disease. J Food Protect 1995; 58:927–30.
156. Survey of consumer food handling practices and awareness of microbiological hazards. Washington, DC: United States Department of Agriculture, Food Safety and Inspection Service, Policy Analysis Unit, 1994.
157. Meat and poultry facts. Arlington, VA: American Meat Institute, 1994.
158. Putnam JJ, Allshouse J. Food consumption, prices, and expenditures. Washington, DC: United States Department of Agriculture, 1994. (Statistical bulletin no. 915).
159. Duewer LA. Changing trends in the red meat distribution system. Washington, DC: United States Department of Agriculture, 1984. (Statistical bulletin no. 509).
160. Clarke R, McEwen S, Harnett N, et al. The prevalence of verotoxin-producing *Escherichia coli* (VTEC) in bovines at slaughter. In: Abstracts of the Annual Meeting of the American Society of Microbiologists, Miami Beach, Florida, May 8–13, 1988. Washington, DC: American Society for Microbiology, 1988:282.
161. Montenegro MA, Bulte M, Trumpf T, et al. Detection and characterization of fecal verotoxin-producing *Escherichia coli* from healthy cattle. J Clin Microbiol 1990;28:1417–21.
162. Blanco M, Blanco J, Blanco JE, et al. Enterotoxigenic, verotoxigenic, and necrotoxigenic *Escherichia coli* isolated from cattle in Spain. Am J Vet Res 1993;54:1446–51.
163. Martin DR, Uhler PM, Okrend AJG, et al. Testing of bob calf fecal swabs for the presence of *Escherichia coli* O157:H7. J Food Protect 1994;57:70–2.
164. *Escherichia coli* O157:H7 in US dairy calves. Washington, DC: United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, 1994.
165. Chapman PA, Wright DJ, Norman P. Verotoxin-producing *Escherichia coli* infections in Sheffield: cattle as a possible source. Epidemiol Infect 1989;102:439–45.
166. Synge BA, Hopkins GF. Verotoxigenic *Escherichia coli* O.157 in Scottish calves. (Letter). Vet Rec 1992;130:583.
167. Chapman PA, Siddons CA, Wright DJ, et al. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. Epidemiol Infect 1993;111:439–47.
168. Okrend AJG, Rose BE, Lattuada CP. Use of 5-bromo-4-chloro-3-indoxyl-beta-D-glucuronide in MacConkey sorbitol agar to aid in the isolation of *Escherichia coli* O157:H7 from ground beef. J Food Protect 1990;53:941–3.

169. Thompson JS, Hodge DS, Borczyk AA. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157. J Clin Microbiol 1990;28:2165–8.
170. Chapman PA, Siddons CA, Zadik PM, et al. An improved selective medium for the isolation of *Escherichia coli* O157. J Med Microbiol 1991;35:107–10.
171. Zadik PM, Chapman PA, Siddons CA. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. J Med Microbiol 1993;39:155–8.
172. Szabo RA, Todd ECD, Jean A. Method to isolate *Escherichia coli* O157:H7 from food. J Food Protect 1986;49: 768–72.
173. Okrend AJG, Rose BE, Bennett B. A screening method for the isolation of *Escherichia coli* O157:H7 from ground beef. J Food Protect 1990;53:249–52.
174. Todd EC, Szabo RA, Peterkin P, et al. Rapid hydrophobic grid membrane filter-enzyme-labeled antibody procedure for identification and enumeration of *Escherichia coli* O157 in foods. Appl Environ Microbiol 1988;54:2536–40.
175. March SB, Ratnam S. Latex agglutination test for detection of *Escherichia coli* serotype O157. J Clin Microbiol 1989; 27:1675–7.
176. Okrend AJG, Rose BE, Matner R. An improved screening method for the detection and isolation of *Escherichia coli* O157:H7 from meat, incorporating the 3M Petrifilm (TM) test kit—HEC—for hemorrhagic *Escherichia coli* O157:H7. J Food Protect 1990;53:936–40.
177. Tison DL. Culture confirmation of *Escherichia coli* serotype O157:H7 by direct immunofluorescence. J Clin Microbiol 1990;28:612–13.
178. Samadpour M, Liston J, Ongerth JE, et al. Evaluation of DNA probes for detection of Shiga-like-toxin-producing *Escherichia coli* in food and calf fecal samples. Appl Environ Microbiol 1990;56:1212–15.
179. Fratamico PM, Sackitey SK, Wiedmann M, et al. Detection of *Escherichia coli* O157:H7 by multiplex PCR. J Clin Microbiol 1995;33:2188–91.